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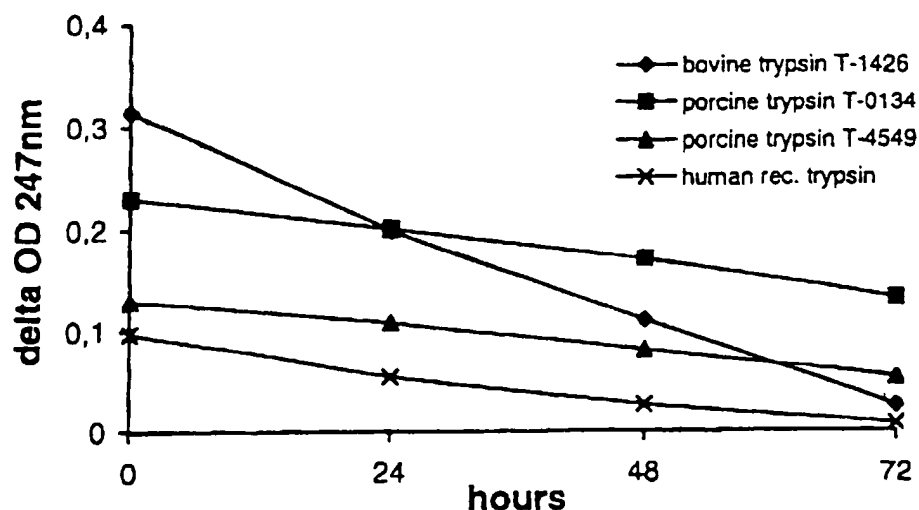
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(54) Title: LIVE VACCINE AND METHOD OF MANUFACTURE



(57) Abstract: The invention relates to a simple and efficient process for isolating viruses from various sources and for producing live attenuated influenza vaccines in a serum-free Vero cell culture under conditions where alterations in the surface antigens of the virus due to adaptive selection are minimized or prevented. The process does not require purification of the virus-containing supernatant harvested from the cell culture nor post-incubation treatment of the viruses for HA activation. The invention further relates to influenza A and B master strain candidates and to vaccines made thereof.

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## LIVE VACCINE AND METHOD OF MANUFACTURE

## TECHNICAL FIELD

5 The present invention is in the field of virology and vaccine development and relates to an improved method of manufacture of a viral vaccine, particularly of a whole-virus vaccine, preferably of an attenuated live vaccine and to vaccines obtainable by the method.

## 10 BACKGROUND OF THE INVENTION

The influenza hemagglutinin (HA) antigen is the major target for the protective immune responses of a host to the virus.

A common practice of recovering new viral isolates involves recovery from a  
15 nasal or throat swab or from a similar source, followed by cultivation of the isolates in embryonated chicken eggs. The virus adapts to its egg host and large scale production of the virus can be carried out in eggs. Such conventional methodology involving embryonated chicken eggs to produce Influenza vaccine is, however, extremely cumbersome, involving the handling of many thousands  
20 of eggs per week as well as extensive purification of the virus suspension derived from the allantoic fluid to ensure freedom from egg protein.

Another disadvantage in the use of chicken embryos for virus production lies in the fact that this substrate strongly favors the selection of virus variants that  
25 differ in their antigenic specificity from the wildtype virus and not rarely results in viruses that may not be suitable for vaccine production due to their altered phenotypes including, for instance, considerable reduction in immunogenicity.

Many attempts have therefore been undertaken in the art to utilize standard  
30 tissue culture technology with established mammalian cell lines, such as MDCK (Madin-Darby Canine Kidney) or Vero (African Green Monkey Kidney) cells, for virus production, particularly influenza virus production.

One of the difficulties in growing influenza strains in tissue cell culture arises  
35 from the necessity for proteolytic cleavage of the influenza hemagglutinin in the host cell. Cleavage of the virus HA precursor into the HA1 and HA2 subfragments, although not necessary for the assembly of the viral elements to

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form a complete virion, is required, however, to render the virion infective, i.e. to enable it to infect a new cell.

It has been reported (e.g. Lazarowitz et al., "Enhancement of the Infectivity of  
5 Influenza and B Viruses by Proteolytic Cleavage of the Hemagglutinin  
Polypeptide", Virology, 68:440-454, 1975) that the limited replication of several  
influenza A strains in standard cell cultures could be overcome by the addition  
of proteases like trypsin to the tissue culture medium. Yet, there remained  
difficulties in some cases, for instance when using Vero cells.

10

Kaverian and Webster (J Virol 69/4:2700-2703, 1995) report that in Vero cell  
cultures, and less pronounced in MDCK, swine kidney, or rhesus monkey kidney  
cell cultures, the trypsin activity in the medium rapidly decreased from the onset  
of incubation resulting in the failure of virus accumulation in the medium due to  
15 the lack of production of a sufficient number of infective virions. They  
concluded that a trypsin inhibiting factor was released from the Vero cells. They  
further showed that by repeated addition of trypsin reproduction of virus could  
be resumed and maintained for a number of reproduction cycles resulting in a  
much better virus yield.

20

Another way for efficient vaccine production was reported in US 5,753,489  
wherein serum-free medium was used for virus propagation in a number of  
different mammalian cells including MDCK and Vero cells. The method disclosed  
therein comprises growing vertebrate cells in serum-free medium, infecting the  
25 cell culture with a virus, incubating the cell culture infected with the virus,  
removing a portion of the virus-containing medium and contacting this portion  
with a protease, thereafter adding to that portion a protease inhibitor and  
returning that portion to the cell culture. It is preferred therein to provide the  
steps of growing, infecting and incubating in a first vessel and the steps of  
30 trypsin-contacting and inhibitor-adding are performed in a second vessel  
connected with the first vessel in a loop so that the steps can be performed in  
a closed cycle. This system allows to use trypsin or other proteolytic enzymes  
at much higher concentrations than those normally tolerated by cells in culture.

35 EP 0870508 reports a method to produce a viral antigen vaccine comprising  
infecting an animal cell line, optionally a Vero cell line, with virus, propagating  
virus in the cell culture, adding a nuclease enzyme to the cell culture shortly

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- before the end of virus propagation to digest nucleic acid material released from the lysing host cells into the medium, harvesting the virus and obtaining viral antigens thereof by extraction in order to make the viral antigen vaccine. The patent is silent with regard to the kind of nutrient medium used for virus
- 5 propagation and also with regard to the addition of a protease, usually required for the final processing of influenza virus hemagglutinin to get infectious virus. The method further requires various purification steps for providing a ready-for-use vaccine preparation.
- 10 It is known, however, that the nature the host substrate as well as the composition of the nutrient medium used for virus propagation may significantly affect immunogenicity and antigenicity of the virus progeny obtained therewith. Particularly, serum-containing media may not only decrease antigenicity of viral progeny but additionally may decrease protease activity in the medium, hence
- 15 inhibit virus maturation, and subsequently require expensive steps of purification.

#### SUMMARY OF THE INVENTION

- 20 The present invention overcomes the drawbacks of the prior art. It relates to a simple and efficient process for isolating viruses from various sources and for producing viral progeny for use as vaccines, particularly live attenuated influenza vaccines, in under conditions where alterations in the surface antigens of the virus due to adaptive selection are minimized or entirely prevented.
- 25 It is also an object of the present invention to provide for a method for the production of viruses, particularly influenza viruses, that yields viral progeny that selectively agglutinates human erythrocytes but not chicken erythrocytes, and that preferably has antigenic properties identical with those of the initially
- 30 inoculated virus strain, e.g. a primary clinical wildtype isolate.

In a preferred embodiment, the nucleic acid sequence of the HA gene and optionally of the NA gene of the propagated virus is identical with the one of the initially inoculated strain (e.g. an epidemic strain, primary clinical isolate of

35 an infected patient).

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It is another object of the invention to provide a method for efficient production of a whole-virus vaccine, particularly a live attenuated vaccine, in a single step procedure that does not require any chromatographic or other purification steps of the virus suspension harvested from the cell culture supernatant by

5 centrifugation, particularly no protein separation or purification steps.

It is yet another object of the invention to provide attenuated, cold adapted and temperature sensitive influenza A and B strains and vaccines made thereof.

## 10 BREF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graphic illustration of the time course of trypsin inactivation in the supernatant of a Vero cell culture.

15 Fig. 2 is a graphic illustration of the time course of trypsin inactivation in the supernatant of a MDCK cell culture.

## DETAILED DESCRIPTION OF THE INVENTION

20 Comparative experiments using embryonated eggs, MDCK and Vero cells clearly proved that the initially inoculated virus is likely to undergo antigenic alteration during growth on any one of these substrates

Our experiments confirmed that the alterations are least or even absent for  
25 influenza virus strains grown on Vero cells in serum-free medium. Moreover, it turned out that influenza A viruses, at least strains of the H3N2 subtype, when multiplied on Vero cells in serum-free and protein-free medium exhibit a selectivity for agglutination of human erythrocytes but not for chicken erythrocytes. Also, they did not grow on eggs. This was a first indication that  
30 these Vero-grown viruses might be more identical with the wildtype virus of the corresponding clinical isolate than the ones grown on MDCK cells or eggs.

Indeed, comparison of the HA and NA gene sequences of wildtype isolates obtained from nasal swabs with the ones of the same viruses after growth on  
35 Vero and MDCK cells, respectively, revealed alterations in the HA or NA of MDCK-grown viruses relative to the HA or NA of the swab isolates or of the Vero-grown viruses or of both the swab isolates and the Vero-grown viruses.

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Moreover, experimental data obtained from immunizations of ferrets with Vero- and MDCK-grown wildtype viruses indicate a far stronger virulence of the Vero-grown viruses compared to the MDCK-grown viruses. Also, the immunogenicity of the Vero-grown viruses tested in an animal trial on macaques was  
5 demonstrated to be significantly superior to the one of the viruses grown on MDCK cells or eggs.

These findings together provide strong evidence for the hypothesis that the process for the multiplication and propagation of viruses according to the  
10 present invention as hereinafter described in more detail yields viruses that are either unaltered compared to the initially inoculated (e.g. wildtype) virus or are modified to only a minor extent.

It is not only the avoidance of antigenic alterations that makes the present  
15 process of virus multiplication so unique, but it is also its striking simplicity which makes it extremely suitable for large scale industrial vaccine production.

Further experiments have shown that the source of trypsin (or trypsinogen) may be one additional factor that influences the overall yield of infective virions.  
20 Indeed, while the methods known in the art (e.g. Kaverin and Webster, J Virol 69/4:2700-2703, 1995; or US 5,753,489) use either repeated addition of trypsin (Kaverin and Webster) or high trypsin concentrations (US 5,753,489), the process according to the present invention applies only half or less of the trypsin concentrations reported in the prior art. Moreover, a single addition of as  
25 little as 0.5 - 10 µg, preferably 2 - 5 µg trypsin per ml to the cell culture medium prior to or at the beginning of incubation of the infected host cells is sufficient to reach optimal infective virus titers. Inactivation experiments revealed that porcine or human recombinant trypsins are far less susceptible to inactivation by Vero or MDCK cells than bovine trypsin. Since bovine trypsin is  
30 most commonly used in the art it is rather likely that prior art literature unless explicitly mentioning another trypsin source, implicitly refers to bovine trypsin only. This would also help to explain the modes and concentrations of trypsin application recited, for instance, in Kaverin et al. and in US 5,753,489.

35 Using porcine or human rec trypsin or trypsinogen for initially supplementing the serum-free medium for Vero cell cultures according to the present invention therefore allows to use extremely low trypsin or trypsinogen concentrations and

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thus prevents the need of labor-intensive and costly purification steps after harvesting of the virus-containing supernatant.

Another step that contributes to make the present process simple and therefore  
5 attractive to vaccine manufacturers is the addition of a single dose of highly active endonuclease to the cell culture medium prior to or at the beginning of incubation of the infected Vero cells for virus propagation. This endonuclease, preferably Benzonase<sup>TM</sup>, is added once to the medium at a very low initial concentration of 2 - 30, preferably 5 - 15, Units per ml of medium and  
10 effectively clears the cell culture medium from free DNA and RNA originating mainly from the lysing or lysed host cells. The residual Benzonase enzyme concentration in the ready-for-use vaccine preparations obtained from the centrifuged supernatant remains at 5 ng or less per dose.

15 Benzonase<sup>TM</sup> is a trademark of Nycomed Pharma A/S Denmark and relates to an extracellular unspecific endonuclease obtained from *Serratia marcescens*. Benzonase is a genetically engineered endonuclease which degrades both DNA and RNA strands in many forms to small oligonucleotides. It promotes quick reduction of the viscosity of cell lysates, which facilitates ultracentrifugation. It  
20 reduces proteolysis and increases the yield in targeted protein and offers complete elimination of nucleic acids from, e.g. recombinant, proteins. It has an exceptionally high activity of 400,000 U/mg.

A third and important advantage of the present process is the factor time hence  
25 process costs. Due to the use of serum-free medium that does not contain proteins of animal origin and preferably no antibiotics, expensive and time-consuming purification procedures can be reduced to a minimum or even totally avoided. Also, because the addition of exogenous enzymes such as the protease (e.g. trypsin or trypsinogen) and nuclease (e.g. Benzonase) occurs  
30 once at the beginning of the virus propagation phase this saves plenty of time that the state-of-the-art methods require for post-incubation treatment of the virus-containing culture supernatant (e.g., HA activation, RNA/DNA digestion, protein purification, etc.).

Surprisingly, it turned out that the early addition of either or both of protease  
35 (e.g. trypsin or trypsinogen) and nuclease (e.g. Benzonase) to the virus-infected Vero-cell culture had no negative implications on the virus yield, which is



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probably due to the very low enzyme concentrations applicable in the process of the present invention.

The present process of virus propagation is useful for the multiplication of  
5 various kinds of viruses, particularly influenza A viruses of the H3N2 subtype, but is also suitable for the isolation and reproduction of any epidemic or laboratory influenza virus strain, regardless of the kind of virus inoculum (e.g., blood serum sample, nasal wash, nasal swab, pharyngeal swab, saliva, etc.). Using the principles of this process, a number of influenza A and B vaccines has  
10 been produced which are part of the present invention and which are characterized in more detail in the subsequent Examples.

Also, protective efficacy as well as vaccine safety have been confirmed for the vaccines made according to the present invention, as will be demonstrated in the Examples.

15

The term "protein-free" or "free of non-serum proteins" as used herein in connection with the method of virus multiplication or propagation according to the present invention shall mean free of any functionally active protein. It shall not exclude, however, non-functional peptides as may originate from protein  
20 hydrolysates such as yeast extract or soya extract. Unless stated otherwise, the term "protein-free" shall neither exclude the presence of a protease and a nuclease enzyme at the concentrations disclosed and claimed herein.

In a preferred embodiment, the present invention relates to a simple, reliable  
25 and highly economic method for the manufacture of a whole-virus vaccine, preferably of an attenuated live vaccine, comprising the steps of:

- a) infecting African Green Monkey Kidney (Vero) cells with a desired virus, wherein the Vero cells have been grown in and separated from a serum-free medium that is also free of non-serum proteins;
- 30 b) combining the infected cells with a suitable serum-free cell culture medium that is also free of non-serum proteins except for a protease and a nuclease; and
- c) incubating the cells in the presence of said protease and said nuclease to allow for production of infectious virus and, simultaneously, for digestion of  
35 nucleic acid material released to the cell culture medium;
- d) harvesting infectious virus by collecting virus-containing supernatant obtained from centrifugation of the cell culture; and

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e) preparing a vaccine thereof comprising subjecting the virus-containing supernatant to at least one processing step selected from the group consisting of filtering, concentrating, freezing, freeze-drying, and stabilizing by addition of a stabilizing agent.

5

It is preferred that the virus used for propagation has never had any contact to a host substrate other than a Vero cell line. This will ensure best results with regard to immunogenic and antigenic identity of the initial virus (e.g. nasal swab isolate) and the viral progeny obtained after propagation.

10

It is also preferred that the virus used for propagation, particularly for the manufacture of a whole-virus vaccine, preferably an influenza attenuated live vaccine, is an influenza virus selected from the group consisting of strains A/Sing/1/57ca, A/Sing/1/57ca/ $\Delta$ NS 87, A/Sing/1/57ca/ $\Delta$ NSPR8,

15 A/Sing/1/57ca/NS124PR8, B/Vienna/1/99ca, B/Vienna/99/ca37 and any attenuated variants and reassortants derived from any one of these strains. The genetic characteristics of the preferred virus strains, e.g. master strains, are disclosed in full detail in the subsequent Examples.

20 In another embodiment, the present invention refers to a whole-virus vaccine itself, preferably to an attenuated live vaccine, which in its ready-for-use form comprises essentially unmodified, optionally filtered and/or concentrated, virus-containing supernatant of a serum-free and protein-free Vero cell culture used for production of said virus. It is particularly preferred that the vaccine is  
25 produced according to the method of the present invention as disclosed and claimed herein.

This "one-step" vaccine, which does not require further processing, e.g., purification steps other than centrifugation and/or conventional filtration (i.e. not gel filtration), is compliant with the requirements for FDA approval.

30

The term "essentially unmodified" as used herein with regard to virus-containing supernatant in vaccine preparations according to the present invention shall refer to the composition of the supernatant as is at the time of harvesting the propagated virus, i.e. to the composition of the soluble components and  
35 ingredients present in the liquid phase of the supernatant. Minor alterations of the composition of ingredients as may occur due to steps of, for example, filtration, sterile filtration, centrifugation, concentration, drying, or freeze-drying

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of the virus-containing supernatant, shall be regarded as falling within the scope of "essentially unmodified". Also, the term shall not exclude the presence of preserving and/or stabilizing agents usually applied in the art to vaccine preparations.

5

The whole-virus vaccines of the present invention may be used for the prophylactic or therapeutic treatment of viral infections, particularly of influenza virus infections. They may be administered as known in the art, e.g. intravenously, subcutaneously, intramuscularly or, most preferably, intranasally.

10 The virus strains disclosed herein and the vaccines made thereof may, however, also be used as vectors or shuttles to present heterologous antigens to the immune system, e.g. antigens of viral envelope proteins such HIV-1 or hepatitis antigens.

15 Further preferred embodiments are defined in the dependent claims.

In order that the invention described herein may be more fully understood, the following Examples are set forth. They are for illustrative purposes only and are not to be construed as limiting this invention in any respect.

20

Example 1: Virus production

Cultivation of Vero /SF (= serum-free) cells:

25 SF-Medium: DMEM (Biochrom F0435), Ham's F12 (Biochrom F0815), 5mM L-Gln, 0.1% SF-supplement (a) or (b); antibiotics (only for first passage of virus isolation).

SF-Supplement: protein hydrolysate of non-animal origin, without functional proteins such as insulin, transferrin or growth factors:

30 a) 62.5 g hy-soy/UF, Quest 5X59100, to 500 g HQ-water, filtered with PES 0.2 µm filter;  
b) 12.5 g hy-pep 1510, Quest, to 100 g HQ-water, filtered with PES 0.2 µm filter.

35 The content of a deep frozen (liquid nitrogen) disinfected (70% ethanol) ampule of WCB Vero cells was thawed and added to 9 ml of cold serum-free (SF) medium in a 10 ml tube and centrifuged for 10 min at 1000rpm (170 g). The

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pellet was resuspended in SF-medium to a total of 30 ml, transferred to a 80 cm<sup>2</sup> Roux bottle and incubated at 37°C and 7%CO<sub>2</sub> for at least 15 min. Thereafter, the medium was removed and the cells were washed with approx. 0.1 ml/cm<sup>2</sup> PBS def.(= PBS without Ca<sup>2+</sup> and Mg<sup>2+</sup>). Addition of  
5 trypsin/EDTA-solution (8-10 µl/cm<sup>2</sup>; 0.1% trypsin / 0.02% EDTA-solution) and incubation at room temperature for about 3 min. Detaching by gently pushing the Roux bottle against palm of the hand, addition of SF-medium and trypsin inhibitor (Sigma, T6522) at a quantity of about 1/5 of volume of the trypsin/EDTA solution. Repartition of the cell suspension to Roux bottles or  
10 roller bottles, incubation at 37°C and 9% CO<sub>2</sub>.

MDCK cells were grown in DMEM/Ham's F12 + 2% FCS (heat inactivated); embryonated hen eggs were 11-12 days old and of SPF (specific pathogen free) origin.

15

Propagation of virus strains:

Old medium from roller bottles containing Vero cells was removed and cells were infected with virus by addition of 5 ml virus suspension in SF-medium to  
20 each roller bottle, resulting in an MOI (multiplicity of infection) of approximately 0.01. After incubation for 45 minutes at 33°C the virus inoculum was removed with a pipette. 90ml of SF-medium supplemented with 0.5 - 10, preferably 2 - 5 and most preferably 2 µg/ml porcine trypsin (supplier: AvP) or human recombinant trypsin or trypsinogen (own production) and 0.5 g/l sodium  
25 bicarbonate were added to each roller bottle and the bottles incubated at 33°C and 5% CO<sub>2</sub>. For the production of attenuated live vaccine samples for use in animal testing and in human clinical trials the SF-medium was supplemented with trypsin and, additionally, with Benzonase<sup>TM</sup> at a concentration of 2 - 30, preferably 5 - 15, and most preferably 10 Units of Benzonase<sup>TM</sup> per ml of  
30 medium. Virus was harvested after 64 hours post infection by centrifugation of the culture supernatant for 5 min at 4000 rpm (3000g) at 10°C in 50 ml-tubes. The supernatant was pooled for each virus strain and stored at +4°C. Aliquots thereof were used for vaccine testing.

35 For storage purposes the virus preparations may be freeze-dried and stabilizer such as, for example, trehalose and lactalbumin enzymatic hydrolysate in HEPES buffer may be added. Reconstitution may be done with sterile water.

Example 2: Comparison of trypsin inactivation in cell cultures

Table 1: Trypsin inactivation in Vero vs. MDCK cell culture

	Vero / MDCK			
	0 h	24 h	48 h	72 h
bovine trypsin	0.314/0.314	0.199/0.239	0.110/0.201	0.026/0.203
porcine trypsin (high)	0.230/0.230	0.201/0.206	0.171/0.209	0.133/0.201
porcine trypsin (low)	0.129/0.129	0.108/0.118	0.081/0.099	0.054/0.116
human rec trypsin	0.097/0.097	0.054/0.088	0.026/0.080	0.008/0.076

- 5 Supernatants obtained from uninfected Vero cell cultures (grown in SF medium as described in Example 1) and MDCK cell cultures (grown in FCS-supplemented medium as described in Example 1) were tested for their capacity to inactivate trypsin of different origin that has been added to the supernatant at time = 0 h at equal concentrations each. Porcine trypsin has been applied in two different
- 10 qualities (obtained from different manufacturers), i.e. with high or low activity. The results are presented in Table 1 and in Figures 1 and 2.

The data unambiguously show that bovine trypsin is rapidly inactivated in Vero cell culture supernatant and less rapidly in MDCK cell culture supernatant.

- 15 Porcine and human rec trypsin (manufactured in our laboratories) remain fully active in MDCK supernatants while they are gradually inactivated in Vero supernatants at approximately half or less of the velocity of bovine trypsin inactivation. The difference of the porcine trypsins tested is only in the starting OD-level at 247 nm, while the inactivation characteristics are essentially
- 20 identical for both lots of porcine trypsin.

Example 3: Comparison of various viral properties after growth on different host cell substrates

- 25 Virus propagation was carried out as described in Example 1 for the different host cell substrates. Each of the seven isolates recovered on Vero cells was reactive with human erythrocytes but not with chicken erythrocytes and none of them accumulated in embryonated eggs. On the other hand, all isolates recovered on MDCK cells were reactive both with chicken and human
- 30 erythrocytes and were capable of growing in eggs. Although these differences were not seen in influenza A viruses of the H1N1 subtype nor in influenza B

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isolates (see subsequent Tables 3 and 4), it may nevertheless be assumed that cultivation of influenza viruses on Vero cells will maintain antigenic properties more properly than cultivation on other substrates.

5 Table 2: Characteristics of H3N2 viruses isolated from clinical material on Vero/SF cells

Isolate number	Antigenically related to	Isolated on	HA titer with		Growth in eggs
			chicken erys	human erys	
A/47/96	A/Johannesburg/33/94	Vero MDCK	- +	+ +	- +
A/7729/98	A/Sydney/5/97	Vero MDCK	- +	+ +	- +
A/1143/99	A/Sydney/5/97	Vero MDCK	- +	+ +	- +
A/1144/99	A/Sydney/5/97	Vero MDCK	- +	+ +	- +
A/1179/99	A/Sydney/5/97	Vero MDCK	- +	+ +	- +
A/1180/99	A/Sydney/5/97	Vero MDCK	- +	+ +	- +
A/1182/99	A/Sydney/5/97	Vero MDCK	- +	+ +	- +

From the data in Table 3 it appears that H1N1 influenza viruses may be less susceptible to adaptive selection, as the Vero and MDCK-grown isolates do not exhibit significant differences in their hemagglutination characteristics nor in their HA sequences. A similar conclusion may be drawn for the B isolates listed in Table 4.

The clinical starting material (e.g. serum samples, swabs) for virus isolation and replication was primarily obtained from:

- 15 1. Institute of Virology, Vienna, Austria (Prof. F. Heinz) 1995/96, 1996/97
2. Unité de Génétique Moléculaire des Virus Respiratoires, Institute Pasteur, Paris, France (Prof. S. van der Werf) 1996/97
3. Public Health Laboratory Service, London, UK (Dr. M. Zambon) 1996/97
4. Laboratoire Central de Virologie, Hôpitaux Universitaires de Genève, Geneva, Switzerland (Dr. W. Wunderli) 1996/97, 1997/98

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## 5. Virus Unit, Queen Mary Hospital, Hong Kong (Dr. W.L. Lim) 1997/98

Table 3: Characteristics of H1N1 viruses isolated from clinical material on  
Vero/SF cells

Isolate number	Antigenically related to	Isolated on	HA titer with		Growth in eggs	Changes in HA1 at position
			chicken erys	human erys		225
A/5389/95	A/Bayern/7/95	Vero	+	+	+	D
		MDCK	+	+	+	D
A/1035/98	A/Beijing/262/95	Vero	+	+	+	D
		MDCK	+	+	+	D
		Egg	+	+	+	G
		Swab	+	+	+	D
A/1131/98	A/Beijing/262/95	Vero	+	+	+	D
		MDCK	+	+	+	D
		Swab	+	+	+	D
A/1134/98	A/Beijing/262/95	Vero	+	+	+	D
		MDCK	+	+	+	D
		Egg	+	+	+	n.t.
		Swab	+	+	+	D

5

Tabelle 4: Characteristics of B viruses isolated from clinical material on Vero/SF  
cells

Isolate number	Antigenically related to	Isolated on	HA titer with		Growth in eggs	Changes in HA1 at position
			chicken erys	human erys		198
B/4291/97	B/Beijing/184/93	Vero	+	+	+	identical
		MDCK	+	+	+	
B/1/99	B/Beijing/184/93	Vero	+	+	+	T(g.s)
		MDCK	+	+	+	T(g.s)
		EGG	+	+	+	A
		Swab	+	+	+	T(g.s)

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B/110/99	n.t.	Vero	+	+	+	identical
		MDCK	+	+	+	
B/147/99	n.t.	Vero	+	+	+	identical
		MDCK	+	+	+	
B/156/99	B/Beijing/184/93	Vero	+	+	+	identical
		MDCK	+	+	+	
B/157/99	B/Beijing/184/93	Vero	+	+	+	identical
		MDCK	+	+	+	

Table 5: Amino acid changes in HA, NA and M proteins of H3N2 influenza viruses isolated on different host systems

Isolate number	Changes at positions								
	HA						NA		M
	128	129	229	133	218	220	136	151	
A/47/96 Vero	T(g.s)								
A/47/96 MDCK	A								
A/7729/98 Vero		E	R						
A/7729/98 MDCK		G	K						
A/1143/99 Swab				N(g.s)	G		n.t	n.t	n.t
A/1143/99 Vero				N(g.s)	G			D	identical
A/1143/99 MDCK				D	E			G	
A/1144/99 Swab						R	n.t		n.t
A/1144/99 Vero						R	identical		identical
A/1144/99 MDCK						G			
A/1179/99 Swab	identical						n.t		n.t
A/1179/99 Vero							identical		identical
A/1179/99 MDCK									
A/1180/99 Swab	identical						n.t	n.t	n.t
A/1180/99 Vero							Q		identical
A/1180/99 MDCK							R		
A/1182/99 Swab	identical						n.t		n.t
A/1182/99 Vero							n.t		n.t
A/1182/99 MDCK							n.t		n.t

5

The results show that with some isolates there was no alteration of the HA sequence of Vero or MDCK propagated viruses over the HA sequence directly obtained from the swab material by PCR amplification. In some other isolates



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grown on MDCK cells the HA and/or NA sequences were deviating from the corresponding sequences obtained on Vero cells. The Vero-derived viruses did not show, however, any deviations in the HA sequence over the HA sequence of the swab isolates, where determined.

5

Table 6: Immunogenicity of Vero-, MDCK- and Egg-derived viruses for macaques

Animal number	Virus for immunization	Dose, PFU/ml	Serum HI titers
96	A/Vienna/47/96 V	$5 \times 10^4$	256
88	A/Vienna/47/96 V	$5 \times 10^4$	128
15	A/Vienna/47/96 V	$1.0 \times 10^6$	128
95	A/Vienna/47/96 V	$1.0 \times 10^6$	256
93	A/Vienna/47/96 M	$1.0 \times 10^6$	16
128	A/Johannesburg/33/94 E	$5 \times 10^6$	32
110	A/Vienna/157/97 V	$5 \times 10^4$	128
78	A/Wuhan/359/95 E	$5 \times 10^6$	32

The Macaques were immunized i.n. in the absence of anesthesia with 1 ml of virus suspension

10 V – Vero- isolated virus

M - MDCK -isolated viruses

E - egg isolated viruses

Table 7: Virulence of Vero- and MDCK- derived variants of A/Vienna/47/96 wt virus for ferrets

15

Viruses	Virus dose, PFU/ml	Number of animals with fever on day		
		1	2	3
A/Vienna/47/96 Vero	$2 \times 10^2$	FF	FFF	
	$1 \times 10^3$	FFF	FFF	
A/Vienna/47/96 MDCK	$5 \times 10^2$			
	$5 \times 10^3$		FF	
	$5 \times 10^4$	FF	F	F

Animals were immunized i.n. under ether narcosis with 1 ml of virus suspension.

N- normal temperature from 38.1°C to 39.9°C;

F- fever, more than 40.0°C.

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The most surprising, yet important result in Table 6 is the very low immunogenicity of MDCK-derived A/Vienna/47/96 virus compared with the corresponding Vero-derived virus. It is no particular surprise that the egg-derived viruses show only poor immunogenicity.

5

Similarly, the results listed in Table 7 indicate that Vero-derived viruses are less, if at all, altered by adaptive selection on their host substrate in comparison to MDCK-derived viruses. This means that relative to the MDCK-derived viruses the Vero-derived viruses maintain more or even all of the immunologically

10 relevant, particularly antigenic, properties of the original virus.

Example 4: Vaccine production with preferred strains

The process described in Example 1 was also used for the production of vaccine samples for animal testing and human clinical studies. It is understood that the process of virus propagation described therein also encompasses variations that could be suggested or applied by a person of ordinary skills in the art without inventive input and as long as the variations do not change the sense of the present invention as described herein and in the claims.

20

Vaccine samples containing one or more of the preferred influenza A or B wildtype strains, master strains or reassortant strains (that are subsequently described in more detail) were exclusively produced using the continuous Vero cell line as the host cell system (unless for purposes of comparison with samples obtained from other host substrates) in serum-free medium additionally supplemented with the nutritional ingredients and enzymes as described in Example 1.

Some methods suitable for modifying wildtype viruses including the methods of attenuation (e.g., temperature sensitivity), cold adaptation and reassortment are known in the art and extensively reviewed, for instance, in WO 99/64068.

Further characteristics of the two most preferred influenza A and B master strain candidates useful for attenuated live vaccine production, e.g., by 6/2 reassortment with the HA and NA genes of actual epidemic influenza viruses recommended by the WHO, are given in the following Tables 8 - 13.

35

Table 8: Characteristics of master strain candidates for live influenza vaccines

	Influenza A <i>A/Singapore/1/57/ca</i> H2N2	Influenza B <i>B/Vienna/1/99/ca</i>
Passage history	A/Singapore/1/57 wt egg derived H2N2 20 passages at 37°C on Vero/SF cells 25 passages at 25°C on Vero/SF cells	B/Vienna/1/99 wt Vero derived 1 additional passage at 33°C on Vero/SF cells 22 passages at 25°C on Vero/SF cells
Method of attenuation	Serial passages at optimal and suboptimal temperature on heterologous system	Serial passages at optimal and suboptimal temperature on heterologous system
Phenotypic markers	temperature sensitive (ts) cold adapted (ca) very low reproduction in mouse lungs	temperature sensitive (ts) cold adapted (ca) very low reproduction in mouse lungs
Genotypic markers	Mutations: 13 (8 coding) PB2 3 (2 coding) PB1 2 (1 coding) PA 4 (3 coding) NP 1 M 2 (2 coding) NS 1	Mutations: 5 (3 coding) PB2 0 PB1 1 PA 0 NP 2 (1 coding) M 1 NS 1

Table 9: Full Sequence of the 8 genome segments and of the 10 corresponding proteins of strain A/Singapore/1/57/ca

A/Singapore/1/57/ca (H2N2)			
RNA segment	Nucleotide sequence (cDNA)	Protein	Amino acid sequence
1	SEQ ID No. 1	PB2	SEQ ID No. 9
2	SEQ ID No. 2	PB1	SEQ ID No. 10
3	SEQ ID No. 3	PA	SEQ ID No. 11
4	SEQ ID No. 4	HA	SEQ ID No. 12
5	SEQ ID No. 5	NP	SEQ ID No. 13
6	SEQ ID No. 6	NA	SEQ ID No. 14
7	SEQ ID No. 7	M1	SEQ ID No. 15
		M2	SEQ ID No. 16
8	SEQ ID No. 8	NS1	SEQ ID No. 17
		NS2	SEQ ID No. 18

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ca - cold adapted

It shall be noted, however, that the genome segments No. 4 and 6, i.e., the HA and NA genes, are not required to characterize the influenza A master strain

- 5 candidates, because these genes will be exchanged for the corresponding genes of actual epidemic influenza viruses (as mentioned hereinbefore). The features important for the safety of a vaccine, e.g. temperature sensitivity, or features that allow intranasal administration of a vaccine, namely cold adaptation (because the average temperature in a nose is lower than the usual body
- 10 temperature), are primarily caused by mutations in the remaining 6 genome segments.

The following Table 10 lists the mutations in the genome segments of A/Singapore/1/57/ca compared to the corresponding wildtype strain

15 A/Singapore/1/57/wt.

Table 10: Mutations in the genome segments of attenuated, temperature sensitive, cold adapted influenza strain A/Singapore/1/57/ca compared to A/Singapore/1/57/wt strain

RNA segment	Length (n'ds)	Nucleotides changed			Protein	Length (aa)	Amino acids changed		
		position	wt	ca			position	wt	ca
1	2341	252 581* 1046*	a t g	g c t	PB2	771	- 185 340	- I R	- T I
2	2341	1279* 1965	t a	a c	PB1	757	419 -	L -	I -
3	2233	707* 1425 1537* 1819*	a t a g	t a g c	PA	716	228 - 505 598	I - V Q	N - I E
5	1565	210	g	a	NP	506	-	-	-
7	1027	327* 499*	g g	a c	M1 M2	252 97	101 158 -	R Q -	K R -
8	890	813	a	g	NS1 NS2	237 121	- -	- -	- -

20 Total number of mutations - 13 (8 coding)

\* coding mutations

Preferred variants of A/Sing/1/57/ca comprise the ones listed in the following Table 11, wherein "Δ" means "del" or "delta" and stands for a mutant that contains at least one "deletion" in its NS gene segment.

5 Table 11: Preferred variants of A/Sing/1/57/ca

	A/Sing/1/57/ca	Sing ca/ ΔNS 87	Sing ca/ ΔNSPR8	Sing ca/ NS124PR8
PB2 (Sing ca*)				
PB1 (Sing ca*)				
PA (Sing ca*)				
HA				
NP (Sing ca*)				
NA				
M1,2 (Sing ca*)				
NS1,2 (Sing ca*)				
NS1,2 (PR8**)				
<b>Phenotypes</b>				
ca	+	+	+	+
ts	+	+	+	+
IFN-induct.	—	+ / —	+	+
IFN-sensit	—	+	+	—

\* genome segment originating from A/Singapore/1/57/ca

\*\* genome segment originating from influenza A/PR8/34

ca - cold adapted; ts - temperature sensitive;

aa - amino acid(s)

10 IFN-induct. - strain causes interferon release in host substrates that are able of IFN production, as well as in animal or human immune systems upon administration.

IFN-sensit. - strain is sensitive towards interferon; replication in IFN producing systems is reduced or stopped.

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Sing ca/ $\Delta$ NS 87 - strain A/Singapore/1/57/ca containing deletion of 87 amino acids in NS1 gene at aa position 36-123.

Sing ca/ $\Delta$ NSPR8 - strain A/Singapore/1/57/ca containing the NS gene segment from A/PR8/34 (herein also abbreviated "PR8") which contains a deletion of the entire NS1 gene.

Sing ca/NS124PR8 - strain A/Singapore/1/57/ca containing the NS gene segment from A/PR8/34 which contains a stop codon at aa position 124 of the NS1 gene.

+/- means that the phenotype needs further clarification and can not yet be unambiguously defined.

The following Tables 12, 13 and 13A refer to preferred influenza B master strain candidates and to variations and reassortants, respectively, thereof.

Table 12: Full Sequence of the 8 genome segments and of the 11 corresponding proteins of strain B/Vienna/1/99/ca

B/Vienna/1/99/ca			
RNA segment	Nucleotide sequence (cDNA)	Protein	Amino acid sequence
1	SEQ ID No. 19	PB2	SEQ ID No. 27
2	SEQ ID No. 20	PB1	SEQ ID No. 28
3	SEQ ID No. 21	PA	SEQ ID No. 29
4	SEQ ID No. 22	HA <sub>0</sub>	SEQ ID No. 30
5	SEQ ID No. 23	NP	SEQ ID No. 31
6	SEQ ID No. 24	NB	SEQ ID No. 32
		NA	SEQ ID No. 33
7	SEQ ID No. 25	M1	SEQ ID No. 34
		BM2	SEQ ID No. 35
8	SEQ ID No. 26	NS1	SEQ ID No. 36
		NS2	SEQ ID No. 37

ca - cold adapted

The original strain B/Vienna/1/99 was isolated on Vero cell culture grown with serum-free medium in February 1999 in Vienna, Austria from a 12 year old female with acute influenza. It was rated as B/Beijing/184/93-like by the Center for Disease Control (CDC), Atlanta, USA. After an additional passage at 33°C the wildtype strain - designated as B/Vienna/1/99 wt - was attenuated by 22

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serial passages at 25°C using the same cell culture system. The plaque purification was done at 25°C for the first and at 33°C for the following four rounds. The derived plaque purified clone was amplified and stored at -70°C, designated as B/Vienna/1/99 ca or briefly BV22. The identity as a

5 B/Beijing/184/93-like virus was confirmed by HI-assay with standard anti-serum from NIBSC.

Table 13: Mutations in B/Vienna/1/99/ca (= BV22) compared to B/Vienna/1/99/wt (BVie) 1. passage on Vero/SF

Segment (length in nucleotides)	Nucleotides changed			Protein (length in amino acids)	Amino acids changed		
	Posi- tion	BVie	BV22		Posi- tion	BVie	BV22
1 (2396)	-	-	-	PB2 (770)	-	-	-
2 (2369)	594	T	C	PB1 (752)	-	-	-
3 (2305)	-	-	-	PA (726)	-	-	-
4 (1882)	457 1299 1595	G G G	A T A	HA <sub>0</sub> (584)	142 422 521	A K G	T N E
5 (1844)	128 330	C T	T C	NP (560)	23 -	S -	F -
6 (1557)	- 823 1135	- G T	- A C	NB (100) NA (466)	- 257 361	- R I	- Q T
7 (1190)	- 831	- A	- G	M1 (248) BM2 (109)	- 21	- M	- V
8 (1097)	116 -	G -	A -	NS1 (281) NS2 (122)	25 -	A -	T -

10

Table 26: Characterization of B/Vienna/1/99 wt according to Los Alamos National Library influenza database (db) (Web-adress: [www.flu.lanl.gov](http://www.flu.lanl.gov))

B/Vienna/1/99 wt gene coding for	Accession Nr. amino acid seq.	Accession Nr. nucleotide seq	Remarks
PB2, segment 1	ISDACH017	ISDNCHB017	in db listed as segment 2
PB1, segment 2	ISDACH016	ISDNCHB016	in db listed as segment 1
PA, segment 3	ISDACH015	ISDNCHB015	
HA, segment 4	ISDACH018	ISDNCHB018	
NP, segment 5	ISDACH013	ISDNCHB013	
NA, segment 6	ISDACH012	ISDNCHB012	
M, segment 7	ISDACH011	ISDNCHB011	
NS, segment 8	ISDACH014	ISDNCHB014	

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In addition, further passaging of strain B/Vienna/1/99 ca for 15 additional passages (i.e. a total of 37 passages on serum-free Vero cell culture) resulted in a mutant B/Vienna/1/99 ca37 (abbreviated BV37) with properties even superior to the ones of BV22. This mutant contains an increased number of mutations

5 vis-à-vis BV22 and appears to be the currently most promising candidate for the production of a whole-virus vaccine, particularly for an attenuated influenza live vaccine, based on a non-recombinant influenza virus mutant. The additional mutations are listed in Table 13A below:

Table 13 A: Mutations for BV22 and BV37 compared to B/Vienna/1/99 wt 1st  
10 passage on Vero/SF

Segment (length in nucleotides)	Nucleotides changed				Protein (length in amino acids)	Amino acids changed			
	Pos.	BVie	BV22	BV37		Pos.	BVie	BV22	BV37
1 (2396)	-	-	-	-	PB2 (770)	-	-	-	-
2 (2369) (BV37: 2370)	594 2348	T -	<u>C</u> -	<u>C</u> <u>A</u>	PB1 (752)	- -	- -	- -	- -
3 (2305)	-	-	-	-	PA (726)	-	-	-	-
4 (1882)	457 1122 1299 1595	G C G G	A* C <u>T</u> <u>A</u>	A* <u>A</u> <u>G</u> <u>A</u>	HA <sub>0</sub> (584)	142 363 422 521	A F K G	T <sup>+</sup> <u>F</u> <u>N</u> <u>E</u>	T <sup>+</sup> <u>L</u> <u>K</u> <u>E</u>
5 (1844)	128 212 330	C C T	<u>T</u> <u>C</u> C <sup>#</sup>	<u>T</u> <u>T</u> C <sup>#</sup>	NP (560)	23 51 -	S P -	<u>F</u> <u>P</u> -	<u>F</u> <u>L</u> -
6 (1557)	- 823 1135	- G T	- <u>A</u> C <sup>•</sup>	- G C <sup>•</sup>	NB (100) NA (466)	- 257 361	- R I	- <u>Q</u> <u>T</u> <sup>•</sup>	- R T <sup>•</sup>
7 (1190)	24 831 831 1029	G A A A	G <u>G</u> <u>G</u> <u>A</u>	<u>A</u> <u>G</u> <u>G</u> <u>G</u>	M1 (248)  BM2 (109)	- - 21 87	- - M I	- - <u>V</u> <u>I</u>	- - <u>V</u> <u>V</u>
8 (1097)	116 -	G -	<u>A</u> -	<u>A</u> -	NS1 (281) NS2 (122)	25 -	A -	<u>T</u> -	<u>T</u> -

Comparison with influenza sequence database 13.2. 2001 (www.flu-lanl.gov):

a) unique mutations underlined in bold type;

b) mutations common with:

\* B/Lee/40, B/Osaka/70, B/Kadoma/1076/99 (resulting amino acid: I)

15 + B/Lee/40, B/Osaka/70



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# often: B/Lee/40, B/Ann Arbor/1/66 ca & wt, B/Singapore/222/79, B/North Dakota/83, B/Norway/1/84, B/Ibaraki/2/85, B/Ann Arbor/1/86, B/Victoria/2/87, B/Aichi/5/88

• B/Kanagawa/73

- 5 It shall be understood that the influenza A and B master strains according to the present invention shall not be limited to the features and genetic characteristics explicitly listed in the tables herein but shall also comprise minor variations thereof as long as such variations are in the sense of the present invention and do not substantially alter any one of the functional features of the virus.
- 10 Such variations may occur, for instance, due to additional steps of virus multiplication or propagation (e.g. for the purpose of obtaining material for sequence analyses).

Moreover, the gene sequences listed herein include the primer sequences (located at the beginning and at the end of each genome segment) that were  
 15 used along with the present invention, which primer sequences may differ from the corresponding true sequences of the viral genome segments of either or both the wildtype and the attenuated virus strains.

#### Example 5: Vaccine safety and efficacy

20

The subsequent data confirm temperature sensitivity and vaccine safety for influenza vaccines manufactured according to the present invention, e.g., as described in Example 1.

25 Table 14: Antibody response of mice after one intranasal immunisation without narcosis

Viruses	Number of responders <sup>1</sup>	GMT <sup>3</sup>	Protection after challenge <sup>2</sup>
PR8/Sing ca -2/6	0/6	< 4	5/6
PR8/Sing ca -ΔNS	4/6	6.7	5/6
PR8-wt	5/6	16.0	5/6

1 - number of animals with positive HI titer > 1:4

2 - number of animals without detectable virus in the lungs

3- Geometric mean titer of antibodies in serum

30

PR8wt – influenza strain A/PR/8/34 wildtype (H1N1), pathogenic for mice

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PR8/Sing ca-2/6 - is the reassortant between attenuated influenza strain

A/Sing/1/57 ca and PR8 wt, containing 2 genes (HA and NA) from PR8wt virus and all other genes from A/Sing/1/57 ca.

- 5 PR8/Sing-ΔNS contains HA and NA genes from PR8wt, five genes from A/Sing/1/57 ca and the NS gene of PR8 origin lacking the NS1 coding sequence (NS1 deletion or knockout).

Table 15: Antibody response and protection of mice after intranasal immunisation with different variants of A/Singapore/1/57 virus (under narcosis)

Viruses	Responders <sup>1</sup>		GMT after two immunisations	Protection after challenge <sup>4</sup>
	1-st immunisation	2-nd immunisation		
A/Sing/1/57/wt va <sup>2</sup>	9/9	9/9	103.9	9/9
A/Sing/1/57/ca <sup>3</sup>	8/10	10/10	55.7	8/10
A/Sing /57/ΔNS 87	1/10	10/10	27.9	8/10

<sup>1</sup> - number of animals with positive HI titer > 1:4

<sup>2</sup> - va- Vero-adapted

<sup>3</sup> - ca - cold-adapted

<sup>4</sup> - number of animals without detectable virus in the lungs

Table 16: Reproduction of wt, va and ca variants of A/Singapore/1/57 in mouse lungs<sup>a</sup>

Viruses	Virus titer in mouse lungs post infection on day, PFU/ml <sup>b</sup>		
	2	4	6
A/Singapore/1/57/wt	1.6x10 <sup>6</sup>	2.2x10 <sup>5</sup>	1.4x10 <sup>3</sup>
A/Singapore/1/57/wt va	2.5x10 <sup>6</sup>	2.1x10 <sup>6</sup>	1.0x10 <sup>2</sup>
A/Singapore/1/57/ca	< 10	< 10	< 10

<sup>a</sup> Mice were infected i.n. with 50 µl of virus fluid with a titer 1.0 x 10<sup>6</sup> PFU/ml.

<sup>b</sup> PFU/ml of 10% tissue suspension, titrated on MDCK cells.

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Table 17: Virulence of wt and ca variants of A/Singapore/1/57 virus for ferrets

Viruses	Number of animals with fever post infection on day		
	1	2	3
A/Singapore/1/57 wt	FFF	NNN	NNN
A/Singapore/1/57 ca	NNN	NNN	NNN

Rectal temperature of animals was recorded twice a day and characterized as follows:

5 **N** - normal temperature from 38.1 °C to 39.9 °C

**F** - fever, more than 40.0 °C.

Each group consisted of 3 animals, which were immunized i.n. under ether narcosis with 1 ml of virus fluid with a titer of  $2 \times 10^6$  PFU/ml.

10 Table 18: Reproduction of 2/6 reassortant of A/Hong Kong/1035/98 wt and A/Singapore/1/57/ca in mouse lungs<sup>a</sup>

Viruses	Virus titer in mouse lungs on day 2-6 post infection, PFU/ml <sup>b</sup>		
	2	4	6
A/Hong Kong/1035/98 wt H1N1	$6.8 \times 10^4$	$2.0 \times 10^4$	< 10
A/Singapore/1/57/ca x A/Hong Kong/1035/98 wt	< 10	< 10	< 10

<sup>a</sup> Mice were infected i.n. under ether narcosis with 50  $\mu$ l of virus fluid.

<sup>b</sup> PFU/ml of 10% tissue suspension, titrated on Vero/SF cells, data are given as

15 mean value for 6 mice (the lungs of each animal were treated separately).

The reassortant contains the HA and NA genes from A/Hong Kong/1035/98 wt wildtype and the other 6 genes from A/Singapore/1/57/ca.

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Table 19: Virulence of 6/2 reassortant of A/Vienna/47/96 wt and A/Singapore/1/57/ ca for ferrets

Viruses	Virus subtype	Number of animals with fever on day			
		1	2	3	Rhinitis <sup>b</sup>
<i>Master strain</i> A/Singapore/1/57/ ca	H2N2	NNN	NNN	NNN	<u>±</u>
<i>Epidemic virus</i> A/Vienna/47/96 wt	H3N2	NNN	FFF	FFF	+++
<i>Reassortant</i> A/Singapore/1/57/ca x Vienna/47/ 96 wt	H3N2	NNN	NNN	NNN	<u>±</u>

Animals were immunized i.n. under ether narcosis with 1 ml of virus,  $2 \times 10^6$  PFU/ml.

5 N- normal temperature from 38.1 °C to 39.9 °C;

F- fever, more than 40.0 °C.

<sup>b</sup> +++ - severe rhinitis

± absence of rhinitis

- 10 The results presented in Tables 16 to 19 clearly demonstrate the safety of the vaccines containing the attenuated, temperature sensitive master strain or, in case of reassortants, of the vaccines based on the reassorted viruses composed of the "backbone" of the attenuated, temperature sensitive master strain (6 genes) and the HA and NA genes from, e.g., the pathogenic wildtype strain
- 15 A/Hong Kong/1035/98 wt.

Table 20: Ts and ca phenotype of B/Vienna/1/99

Virus	PFU/ml on Vero cells at	PFU/ml on MDCK cells at	
	25 °C	33 °C	39 °C
B/Vienna/1/99 wt	< 300	$4 \times 10^6$	$4 \times 10^5$
B/Vienna/1/99 ca (BV22)	$1 \times 10^6$	$2.4 \times 10^6$	< 20

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Table 21: Genetic stability of the ts phenotype of B/Vienna/1/99 ca

Virus	PFU/ml on MDCK cells at	
	33°C	39°C
B/Vienna/1/99 wt	$4 \times 10^6$	$4 \times 10^5$
B/Vienna/1/99 ca (BV22)	$2.4 \times 10^6$	< 20
B/Vienna/1/99 ca (BV22) after 5 passages at 33°C	$8 \times 10^5$	< 20

The strain BV22 was passaged five times at high MOI on Vero cells. Then the ts-phenotype was controlled again. The strain remained temperature sensitive as can be seen in Table 21.

5

Table 22: Virulence of B/Vienna/1/99 ca and wt in mouse lungs

		PFU/ml* at day post infection		
Virus	organ	2	3	4
B/Vienna/1/99 ca (BV22)	lung	< 20	< 20	< 20
	nose	$1 \times 10^2$	$1 \times 10^2$	20
B/Vienna/1/99 wt	lung	$8 \times 10^4$	$7 \times 10^3$	$4.4 \times 10^3$
	nose	$3.8 \times 10^4$	$3.4 \times 10^4$	$1.4 \times 10^4$

\* 9 OF1 mice per strain were immunized intranasally under ether narcosis with  $10^5$  PFU. At the indicated days post infection 3 mice per group were sacrificed. Lungs and nasal turbinates were homogenized for a 10% (w/v) suspension in PBS def. A plaque assay of the suspensions was performed.

10

The data show that moderate reproduction of the ca master strain candidate BV22 was possible in the nasal mucosa while the ts property of the virus prevented reproduction in the lungs.

15

Table 23: Ts and ca phenotype of the reassortant influenza B strain

Virus	PFU/ml on MDCK cells at	
	33°C	39°C
B/Vienna/1/99 wt	$4 \times 10^6$	$4 \times 10^5$
B/USSR/69 wt	$1.6 \times 10^6$	$4 \times 10^4$
B/Vienna/1/99 ca (BV22)	$1.4 \times 10^6$	< 20
BV22 x B/USSR/69 (6/2)	$8 \times 10^6$	< 20

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A 6/2 reassortant strain containing HA and NA of the wild type influenza strain B/USSR/69 wt and the other 6 genome segments from B/Vienna/1/99 ca (BV22) was established. The origin of the hemagglutinin was tested by HI-assay, all other genome segments by RT-PCT and restriction analysis using 5 methods known in the art.

Table 24: Virulence of the reassortant influenza B strain in mouse lungs

Virus	organ	PFU/ml* at day post infection		
		2	3	4
B/Vienna/1/99 ca (BV22)	lung	< 20	< 20	< 20
	nose	< 20	$1 \times 10^2$	40
B/USSR/69 wt	lung	$1.8 \times 10^5$	$4 \times 10^5$	$2.4 \times 10^4$
	nose	$1.6 \times 10^5$	$2 \times 10^5$	$1.6 \times 10^5$
BV22 x B/USSR/69 wt (6/2)	lung	< 20	< 20	< 20
	nose	$2.8 \times 10^3$	$2 \times 10^3$	$4 \times 10^2$

\* 9 OF1 mice per strain were immunized intranasally under ether narcosis with  $10^5$  PFU. At the indicated days post infection 3 mice per group were sacrificed. Lungs and nasal turbinates were homogenized for a 10% (w/v) suspension in PBS def. A plaque assay of the suspensions was performed.

#### Example 6: Clinical study

15 The following vaccines (in the form of nasal sprays) were produced according to the present invention (e.g. as described in Example 1) for intranasal delivery.

Composition per ml (after reconstitution of freeze-dried material):

- (1) Placebo: 2x SF-medium, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;
- 20 (2) Vero-Vac H1: A/Beijing/262/95 (H1N1)-like preparation comprising  $4.3 \times 10^7$  TCID<sub>50</sub> of 6/2 reassortant A/Singapore/1/57/ca with A/Hong Kong/1035/98; 2x culture supernatant, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;
- (3) Vero Vac H3: A/Sidney/5/97 (H3N2)-like preparation comprising  $2.1 \times 10^7$  TCID<sub>50</sub> of 6/2 reassortant A/Singapore/1/57/ca with A/SW/7729/98; 2x culture supernatant, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;
- 25 (4) Russian trivalent vaccine (live influenza vaccine for adults):  
A/17/Beijing/95/25 (H1N1)  $1.1 \times 10^8$  EID<sub>50</sub>

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- |  |                          |                   |                   |
|--|--------------------------|-------------------|-------------------|
|  | A/17/Sidney/97/76 (H3N2) | $2.3 \times 10^7$ | EID <sub>50</sub> |
|  | B/60/Petersburg/95/20    | $1.1 \times 10^7$ | EID <sub>50</sub> |
- (5) Monovalent Vero vaccine BV22: B/Beijing/184/93 - like preparation comprising  $2 \times 10^6$  TCID<sub>50</sub> of master strain candidate B/Vienna/1/99/ca (= BV22); 2x culture supernatant, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;

The vaccines were administrated to 13 volunteers per each vaccination group. 550 µl of reconstituted vaccine (or placebo, respectively) were given intranasally to each patient on day 0 and for a second time on day 22 ± 1. The results are summarized in Table 25 below.

#### Safety results:

The total number of adverse events (AE) during five days after the first and second vaccination was 14 including 9 mild and 4 moderate AE. Only one volunteer showed severe AE, comprising an increase in body temperature up to 38.8°C within 3 hours after the first vaccination without any local or systemic symptoms. During the next four hours his temperature became normal again. After the first vaccination 7 AE were observed. One of them was local and six were systemic. After the second vaccination 2 local and 5 systemic AE were observed.

No significant difference in terms of safety was revealed between the groups of the study including the one with placebo. No serious AE related to the vaccination were observed except for the one mentioned above. Two of the moderate AE occurred in the H3N2 group (temperature elevation up to 37.6° and acute pharyngitis on day 3 in one volunteer; nasal obstruction, discomfort in the throat on day 22-24 and temperature elevation up to 37.5°C in another volunteer), and one in the H1N1 group (pain in the throat, rhinitis from day 22-26, temperature elevation up to 37 - 37.8°C between days 22-24).

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Table 25: Response of seronegative volunteers to Vero Vac vaccines and to a trivalent Russian cold-adapted egg derived vaccine

No	Vaccine for immunization	Virus dose, TCID <sub>50</sub> /ml or EID <sub>50</sub> /ml	No. of volunteers	% of volunteers with at least 4-fold increase of serum HAI antibody titre to antigens		
				H1N1	H3N2	B
1	Placebo		13		(8)	
2	Vero Vac H1 (H1N1)	4.3x10 <sup>7</sup>	13	38		
3	Vero Vac H3 (H3N2)	2.1x10 <sup>7</sup>	13		67	
4	Russian trivalent vaccine:  A/17/Beijing/95/25 H1N1 A/17/Sidney/97/76 H3N2 B/60/Petersburg/95/20	  1.1x10 <sup>8</sup> 2.3x10 <sup>7</sup> 1.1x10 <sup>7</sup>	13	46	8	31
5	Vero vaccine BV22	2x10 <sup>6</sup>	13			33

(8) patient developed spontaneous infection during course of study.

- 5 The results obtained from the clinical study thus confirm a very good safety of the vaccines produced according to the present invention and using the preferred influenza A and B master strain candidates of the present invention.



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## CLAIMS

We claim

1. A method for the manufacture of a whole-virus vaccine, preferably an attenuated live vaccine, comprising the steps of:
  - 5 a) infecting African Green Monkey Kidney (Vero) cells with a desired virus, wherein the Vero cells have been grown in and separated from a serum-free medium that is also free of non-serum proteins;
  - b) combining the infected cells with a suitable serum-free cell culture medium that is also free of non-serum proteins except for a protease and a  
10 nuclease; and
  - c) incubating the cells in the presence of said protease and said nuclease to allow for production of infectious virus and, simultaneously, for digestion of nucleic acid material released to the cell culture medium;
  - d) harvesting infectious virus by collecting virus-containing supernatant  
15 obtained from centrifugation of the cell culture; and
  - e) preparing a vaccine thereof comprising subjecting the virus-containing supernatant to at least one processing step selected from the group consisting of filtering, concentrating, freezing, freeze-drying, and stabilizing by addition of a stabilizing agent.  
20
2. The method according to claim 1, which does not involve a step of protein separation or purification.
3. The method according to claim 1 or 2, which does not involve a step of  
25 chromatographic separation or purification, and preferably does not contain any purification step other than centrifugation and/or filtration.
4. The method according to any one of claims 1 to 3, which comprises at least one step of sterile filtration of the virus-containing supernatant.  
30
5. The method according to any one of claims 1 to 4, wherein the nuclease has DNase and/or RNase activity, and preferably is Benzonase.
6. The method according to any one of claims 1 to 5, wherein the protease  
35 and the nuclease are added to the cell culture medium once prior to or at the beginning of incubation of the infected cells.

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7. The method according to any one of claims 1 to 6, wherein the protease comprises trypsin and/or trypsinogen of human recombinant or porcine origin which is present in the cell culture medium at an initial concentration of 0.5 - 10, preferably 2 - 5 µg per ml medium.
- 5 8. The method according to any one of claims 1 to 7, wherein the cell culture medium comprises nuclease at an initial concentration of 2 to 30, preferably 5 to 15, U per ml of medium.
- 10 9. The method according to any one of claims 1 to 8, wherein the incubation in step (a) is carried out for 10 to 120 minutes, preferably for 30 to 60 minutes.
- 10 10. The method according to any one of claims 1 to 9, wherein the virus is  
15 selected from the group consisting of a wildtype virus, a primary isolate directly obtained from an infected individual, a recombinant virus, an attenuated virus, a Vero adapted virus, a cold-adapted virus, a temperature-sensitive virus, and a reassortant virus.
- 20 11. The method according to any one of claims 1 to 10, wherein the virus is an influenza A virus, preferably of subtype H3N2 or H1N1, or an influenza B virus.
- 25 12. The method according to any one of claims 1 to 11, wherein the virus has an interferon inducing and/or interferon sensitive phenotype.
13. The method according to any one of claims 1 to 12, wherein the virus is an influenza virus selected from the group consisting of strains  
A/Sing/1/57ca, A/Sing/1/57ca/ΔNS 87, A/Sing/1/57ca/ΔNSPR8,  
30 A/Sing/1/57ca/NS124PR8, B/Vienna/1/99ca, B/Vienna/99/ca37 and any attenuated variants and reassortants derived from any one of these strains.
14. A whole-virus vaccine, preferably an attenuated live vaccine, characterized in that in its ready-for-use form it comprises essentially  
35 unmodified, optionally filtered and/or concentrated, virus-containing supernatant of a serum-free and protein-free Vero cell culture used for production of said virus.

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15. The vaccine according to claim 14, characterized in that it selectively agglutinates human erythrocytes but not chicken erythrocytes.

16. The vaccine according to claim 14 or 15, characterized in that it contains  
5 a suitable stabilizing agent.

17. The vaccine according to any one of claims 14 to 16, characterized in that it is in the form of a liquid, freeze-dried or freeze-dried preparation, optionally suitable for intranasal delivery.

10

18. The vaccine according to any one of claims 14 to 17, characterized in that it is a live attenuated vaccine, preferably comprising whole influenza virus.

19. The vaccine according to any one of claims 14 to 18, characterized in  
15 that it comprises at least one influenza virus having a phenotype with one or more characteristics selected from the group consisting of cold adapted, temperature sensitive, interferon inducing, interferon sensitive.

20. The vaccine according to claim 18, wherein the influenza virus is  
20 selected from the group consisting of strains A/Sing/1/57ca, A/Sing/1/57ca/ $\Delta$ NS 87, A/Sing/1/57ca/ $\Delta$ NSPR8, A/Sing/1/57ca/NS124PR8, B/Vienna/1/99ca, and any attenuated variants and reassortants derived from any one of these strains.

25 21. The vaccine according to claim 14, obtainable by a method of manufacture as defined in any one of claims 1 to 13.

22. A whole-virus vaccine, preferably an attenuated live vaccine, comprising at least one influenza virus selected from the group consisting of strains  
30 A/Sing/1/57ca, A/Sing/1/57ca/ $\Delta$ NS 87, A/Sing/1/57ca/ $\Delta$ NSPR8, A/Sing/1/57ca/NS124PR8, B/Vienna/1/99ca, and any attenuated variants and reassortants derived from any one of these strains.

23. The vaccine according to claim 21, characterized in that it selectively  
35 agglutinates human erythrocytes but not chicken erythrocytes.

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24. The vaccine according to claim 22 or 23, obtainable by a method of manufacture according to any one of claims 1 to 13.

25. Use of a vaccine defined in any one of claims 14 to 24 for prophylactic  
5 or therapeutic administration against viral infection.

26. Use of at least one influenza virus selected from the group consisting of strains A/Sing/1/57ca, A/Sing/1/57ca/ $\Delta$ NS 87, A/Sing/1/57ca/ $\Delta$ NSPR8, A/Sing/1/57ca/NS124PR8, B/Vienna/1/99ca, and any attenuated variants and  
10 reassortants derived from any one of these strains, for the manufacture of a vaccine, preferably for the manufacture of a live attenuated influenza vaccine.

1/1

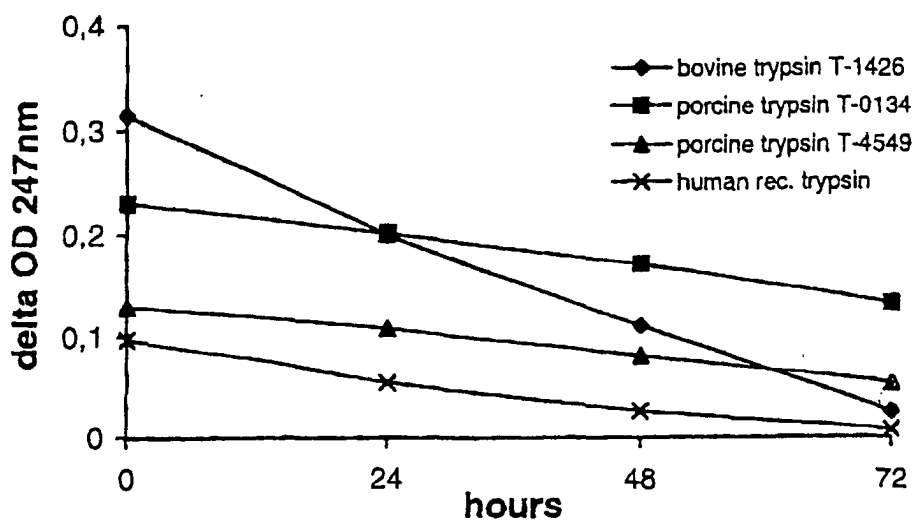


Fig. 1

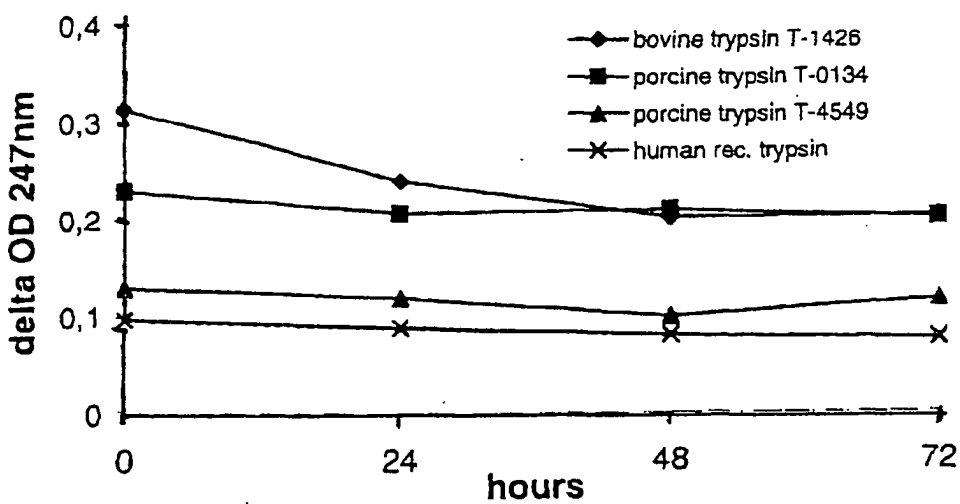


Fig. 2

## SEQUENCE LISTING

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Romanova, Julia

Egorov, Andrej

Ferko, Boris

<120> INFLUENZA VACCINE AND METHOD OF MANUFACTURE

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attggatatg tgggacacca taaattttga gagtactggt aatctaattg caccagagta 840
tggattcaaa atatcgaaaa gaggtaattc agggatcatg aaaacagaag gaacacttga 900
gaactgtgag accaaatgcc aaactccttt gggagcaata aatacaacat tgccttttca 960
caatgtccac cactgacaa taggtgagt ccccaaatat gtaaaatcgg agaagttggt 1020
cttagcaaca ggaccaagga atgttccca gattgaatca agaggattgt ttggggcaat 1080
agctggtttt atagaaggag gatggcaagg aatggttgat ggttggtatg gataccatca 1140
cagcaatgac cagggatcag ggtatgcagc agacaaagaa tccactcaaa aggcatttga 1200
tggaatcacc aacaaggtaa attctgtgat tgaaaagatg aacaccaat ttgaagctgt 1260
tggaagaaag ttacagtaact tagagagaag actggagaac ttgaacaaaa agatggaaga 1320
cgggttttcta gatgtgtgga catacaatgc tgagcttcta gttctgatgg aaaatgagag 1380
gacacttgac tttcatgatt ctaatgtcaa gaatctgtat gataaagtca gaatgcagct 1440
gagagacaac gtcaaagaac taggaaatgg atgttttgaa ttttatcaca aatgtgatga 1500
tgaatgcatg aatagtgtga aaaacgggac gtatgattat cccaagtatg aagaagagtc 1560
taaactaaat agaaatgaaa tcaaaggggt aaaattgagc agcatggggg tttatcaaat 1620

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ccttgccatt tatgctacag tagcagggtc tctgtcactg gcaatcatga tggctgggat 1680  
 ctctttctgg atgtgctcca acgggtctct gcagtgacag atctgcatat gattataagt 1740  
 cttttataa ttaaaaacac ccttgtttct act 1773

<210> 5

<211> 1565

<212> DNA

<213> Influenza virus A/Singapore/1/57/ca

<400> 5

agcaaaagca gggtagataa tcaactcactg agtgacatca aaatcatggc gtcccaaggc 60  
 accaaacggt cttatgaaca gatggaaact gatggggaac gccagaatgc aactgaaatc 120  
 agagcatccg tcgggaagat gattgatgga attggacgat tctacatcca aatgtgcacc 180  
 gaacttaaac tcagtgatta tgaggggcca ctgatccaga acagcttaac aatagagaga 240  
 atggtgctct ctgcttttga cgagaggagg aataaatatc tggaagaaca tccagcgcg 300  
 ggggaaggatc ctaagaaaac tggaggaccc atatacaaga gagtaaatgg aaagtggatg 360  
 agggaactcg tcctttatga caaagaagaa ataaggcgaa tctggcgcca agctaataat 420  
 ggtgatgatg caacagctgg tctgactcac atgatgatct ggcatccaa tttgaatgat 480  
 acaacatacc agaggacaag agctcttgtt cgcaccgga tggatcccag gatgtgctct 540  
 ttgatgcagg gttcgactct ccctaggagg tctggagccg caggcgctgc agtcaaagga 600  
 gttgggacaa tggatgatga gttgatcagg atgatcaaac gtgggatcaa tgatcggaac 660  
 ttctggagag gtgagaatgg gcggaacaac aggattgctt atgagagaat gtgcaacatt 720  
 ctcaaaggaa aatttcaaac agctgcacaa agagcaatga tggatcaagt gagagaaagc 780  
 cggaacccag gaaatgctga gatcgaagat ctcactcttc tggcacggtc tgcactcata 840  
 ttgagagggt cagttgctca caaatcttgt ctgcctgcct gtgtgtatgg aactgccgta 900  
 gccagtgggt acgacttoga aaaagaggga tactctttag tagggataga ccctttcaaa 960  
 ctgcttcaaa acagccaagt atacagccta atcagaccga acgagaatcc agcacacaag 1020  
 agtcagctgg tgtggatggc atgcaattct gctgcatttg aagatctaag agtatcaagc 1080  
 ttcacagag ggaccaaagt aatcccaagg gggaaacttt ccactagagg agtacaatt 1140  
 gcttcaaatg aaaacatgga tactatggaa tcaagtactc ttgaactgag aagcaggtag 1200  
 tgggccataa ggaccagaag tggaggaaac actaatcaac agagggcctc tgcaggtag 1260  
 atcagtgtac aacctacgtt ttctgtgcaa agaaacctcc catttgacaa aacaaccatc 1320  
 atggcagcat tcaactggga tgcagaggga agaacatcag acatgagggc agaaatcata 1380  
 aggatgatgg aagggtgcaa accagaagaa gtgtccttcc agggcgggg agtcttcgag 1440  
 ctctcgagcg aaaaggcaac gaacccgatc gtgccctctt ttgacatgag taatgaagga 1500  
 tcttatttct tcggagacaa tgcagaggag tacgacaatt aaggaaaaat acccttgttt 1560  
 ctact 1565

<210> 6

<211> 1466

<212> DNA

<213> Influenza virus A/Singapore/1/57/ca

<400> 6

agcaaaagca ggagtgaaga tgaatccaaa tcaaaagata ataacaattg gctctgtctc 60  
 tctcaccatt gcaacagtat gcttcctcat gcagattgcc atcctggcaa ctactgtgac 120  
 attgcatttt aaacaacatg agtgcgactc cccgcgagc aaccaagtaa tgccatgtga 180  
 accaataata atagaaagga acataacaga gatagtgtat ttgaataaca ccaccataga 240

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gaaagagatt tgccccgaag tagtggaata cagaaattgg tcaaagccgc aatgtcaaat 300
tacaggatatt gcaccttttt ctaaggacaa ttcaatccgg ctttctgctg gtggggacat 360
ttgggtgacg agagaacctt atgtgtcatg cgatcctggc aagtgttatc aatttgact 420
cgggcagggg accacactat acaacaaaca ttcaaatggc acaatacatg atagaatccc 480
tcatcgaacc ctattaatga atgagttggg tgttccattt catttaggaa ccaaacaagt 540
gtgtgtagca tgggtccagct caagtgtgca cgatggaaaa gcatggttgc atgtttgtgt 600
cactggggat gatagaaatg cgactgctag cttcatttat gacgggaggc ttgtggacag 660
tattggttca tgggtctcaa atatcctcag gaccaggag tcggaatgcg tttgtatcaa 720
tgggacttgc acagtagtaa tgactgatgg aagtgcacaa ggaagagccg atactagaat 780
actattcatt aaagagggga aaattgtccg tattagccca ttgtcaggaa gtgctcagca 840
tatagaggag tgttctgtt accctcgata tcctgacgtc agatgtatct gcagagacaa 900
ctggaaaggc tctaataggc ccgttataga cataaatatg gaagattata gcattgattc 960
cagttatgtg tgctcagggc ttgttggcga cacaccagg aacgacgaca gctctagcaa 1020
tagcaattgc agggatccta acaatgagag agggaaatcca ggagtgaag gctgggcctt 1080
tgacaatgga gatgatgtat ggatgggaag aacaatcaac aaagattcac gctcaggtta 1140
tgaaactttc aaagtcatg gtggttggtc cacacctaat tccaaatcgc aggtcaatag 1200
acaggtcata gttgacaaca ataattggtc tggttactct ggtattttct ctgttgaggg 1260
caaaagctgc atcaataggt gcttttatgt ggagttgata aggggaaggc cacaggagac 1320
tagagtatgg tggacctcaa acagtattgt tgtgtttgtt ggcacttcag gtacttatgg 1380
aacaggctca tggcctgatg gggcgaaat caatttcag cctatataag ctttcgcaat 1440
tttagaaaaa actccttgtt tctact 1466

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&lt;210&gt; 7

&lt;211&gt; 1027

&lt;212&gt; DNA

&lt;213&gt; Influenza virus A/Singapore/1/57/ca

&lt;400&gt; 7

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agcaaaagca ggtagatatt gaaagatgag tcttctaacc gaggtcgaaa cgtatgttct 60
ctctatcgtc ccgtcaggcc ccctcaaagc cgagatcgca cagagacttg aagatgtctt 120
tgctgggaag aacaccgatc ttgaggtctc catggaatgg ctaaagacaa gaccaatcct 180
gtcacctctg actaagggga ttttgggatt tgtattcacg ctcaccgtgc ccagtgcgag 240
aggactgcag cgtagacgct ttgtccaaaa tgccctcaat gggaatgggg atccaaataa 300
catggacaga gcagttaaac tgtataaaaa gcttaagagg gagataacat tccatggggc 360
caaagaaata gcgctcagtt attctgctgg tgcaacttgc agttgtatgg gcctcatata 420
caacaggatg ggggtgtgta ccactgaagt ggcctttggc ctggtatgtg caacctgtga 480
acagattgct gactcccacc ataggtctca taggcaaatg gtgacaacaa ccaatccact 540
aataagacat gagaacagaa tggttctggc cagcactaca gctaaggcta tggagcaaat 600
ggctggatcg agtgagcaag cagcagaggc catggaggtt gctagtcagg ccaggcaaat 660
ggtgcaggca atgagagcca ttgggactca tcctagctcc agtgctggtc taaaagatga 720
tcttcttgaa aatttgcagg cctatcagaa acgaatgggg gtgcagatgc aacgattcaa 780
gtgacctctt tgttgttgc gcgagtatca ttgggatctt gcaactgata ttgtggattc 840
ttgatcgtct ttttttcaaa tgcatttatc gcttctttaa acacggctctg aaaagagggc 900
cttctacgga aggagtacca gagtctatga ggaagaata tcgaaaggaa cagcagagtg 960
ctgtggatgc tgacgatagt cattttgtca gcatagagct ggagtaaaaa actaccttgt 1020
ttctact 1027

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&lt;210&gt; 8

&lt;211&gt; 890

&lt;212&gt; DNA

&lt;213&gt; Influenza virus A/Singapore/1/57/ca

&lt;400&gt; 8

```

agcaaaagca ggggtgacaaa gacataatgg atcctaacac tgtgtcaagc tttcaggtag 60
attgcttcct ttggcatgtc cgcaacaag ttgcagacca agaactaggt gatgccccat 120
tcottgatcg gcttcgccga gatcagaagt ccctaagggg aagaggcagc actctcggtc 180
tgaacatcga aacagccacc cgtgttgga agcagatagt ggagaggatt ctgaaggaag 240
aatccgatga ggcacttaaa atgaccatgg cctccgcacc tgcttcgcga tacctaactg 300
acatgactat tgaggaaatg tcaagggact gggtcatgct aatgccaag cagaaagtgt 360
caggccctct ttgtatcaga atggaccagg caatcatgga taagaacatc atattgaaag 420
cgaatttcag tgtgattttt gaccggctag agaccctaatt attactaagg gctttcaccg 480
aagagggagc aattgttggc gaaatttcac cattgccttc tcttcaggga catactaattg 540
aggatgtcaa aaatgcaatt ggggtcctca tcggaggact tgaatggaat gataacacag 600
ttcgagtctc taaaactcta cagagattcg cttggagaaa cagtaatgag aatgggagac 660
ctccactcac tccaaaacag aaacggaaaa tggcgagAAC aattaggtca aaagttcgaa 720
gaaataagat ggctgattga agaagtgaga cacaattga agataacaga gaatagtttt 780
gagcaaataa catttatgca agccttacag ctgctatttg aagtggaaca agagataaga 840
actttctcgt ttcagcttat ttaatgataa aaaacaccct tgtttctact 890

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&lt;210&gt; 9

&lt;211&gt; 771

&lt;212&gt; PRT

&lt;213&gt; Influenza virus A/Singapore/1/57/ca

&lt;400&gt; 9

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Met Glu Arg Ile Lys Glu Leu Arg Asn Leu Met Ser Gln Ser Arg Thr
  1             5             10             15

Arg Glu Ile Leu Thr Lys Thr Thr Val Asp His Met Ala Ile Ile Lys
      20             25             30

Lys Tyr Thr Ser Gly Arg Gln Glu Lys Asn Pro Ser Leu Arg Met Lys
      35             40             45

Trp Met Met Ala Met Lys Tyr Pro Ile Thr Ala Asp Lys Arg Ile Thr
      50             55             60

Glu Met Ile Pro Glu Arg Asn Glu Gln Gly Gln Thr Leu Trp Ser Lys
      65             70             75             80

Met Asn Asp Ala Gly Ser Asp Arg Val Met Val Ser Pro Leu Ala Val
      85             90             95

Thr Trp Trp Asn Arg Asn Gly Pro Met Thr Ser Thr Val His Tyr Pro
      100            105            110

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Lys Ile Tyr Lys Thr Tyr Phe Glu Lys Val Glu Arg Leu Lys His Gly  
 115 120 125  
 Thr Phe Gly Pro Val His Phe Arg Asn Gln Val Lys Ile Arg Arg Arg  
 130 135 140  
 Val Asp Ile Asn Pro Gly His Ala Asp Leu Ser Ala Lys Glu Ala Gln  
 145 150 155 160  
 Asp Val Ile Met Glu Val Val Phe Pro Asn Glu Val Gly Ala Arg Ile  
 165 170 175  
 Leu Thr Ser Glu Ser Gln Leu Thr Thr Thr Lys Glu Lys Lys Glu Glu  
 180 185 190  
 Leu Gln Asp Cys Lys Ile Ser Pro Leu Met Val Ala Tyr Met Leu Glu  
 195 200 205  
 Arg Glu Leu Val Arg Lys Thr Arg Phe Leu Pro Val Ala Gly Gly Thr  
 210 215 220  
 Ser Ser Val Tyr Ile Glu Val Leu His Leu Thr Gln Gly Thr Cys Trp  
 225 230 235 240  
 Glu Gln Met Tyr Thr Pro Gly Gly Glu Val Arg Asn Asp Asp Val Asp  
 245 250 255  
 Gln Ser Leu Ile Ile Ala Ala Arg Asn Ile Val Arg Arg Ala Ala Val  
 260 265 270  
 Ser Ala Asp Pro Leu Ala Ser Leu Leu Glu Met Cys His Ser Thr Gln  
 275 280 285  
 Ile Gly Gly Thr Arg Met Val Asp Ile Leu Arg Gln Asn Pro Thr Glu  
 290 295 300  
 Glu Gln Ala Val Asp Ile Cys Lys Ala Ala Met Gly Leu Arg Ile Ser  
 305 310 315 320  
 Ser Ser Phe Ser Phe Gly Gly Phe Thr Phe Lys Arg Thr Ser Gly Ser  
 325 330 335  
 Ser Val Lys Ile Glu Glu Glu Val Leu Thr Gly Asn Leu Gln Thr Leu  
 340 345 350  
 Lys Ile Arg Val His Glu Gly Tyr Glu Glu Phe Thr Met Val Gly Lys  
 355 360 365

Arg Ala Thr Ala Ile Leu Arg Lys Ala Thr Arg Arg Leu Ile Gln Leu  
 370 375 380  
 Ile Val Ser Gly Arg Asp Glu Gln Ser Ile Ala Glu Ala Ile Ile Val  
 385 390 395 400  
 Ala Met Val Phe Ser Gln Glu Asp Cys Met Ile Lys Ala Val Arg Gly  
 405 410 415  
 Asp Leu Asn Phe Val Asn Arg Ala Asn Gln Arg Leu Asn Pro Met His  
 420 425 430  
 Gln Leu Leu Arg His Phe Gln Lys Asp Ala Lys Val Leu Phe Gln Asn  
 435 440 445  
 Trp Gly Ile Glu His Ile Asp Asn Val Met Gly Met Ile Gly Val Leu  
 450 455 460  
 Pro Asp Met Thr Pro Ser Thr Glu Met Ser Met Arg Gly Val Arg Val  
 465 470 475 480  
 Ser Lys Met Gly Val Asp Glu Tyr Ser Ser Ala Glu Arg Val Val Val  
 485 490 495  
 Ser Ile Asp Arg Phe Leu Arg Val Arg Asp Gln Arg Gly Asn Val Leu  
 500 505 510  
 Leu Ser Pro Glu Glu Val Ser Glu Thr Gln Gly Thr Glu Lys Leu Thr  
 515 520 525  
 Ile Thr Tyr Ser Ser Ser Met Met Trp Glu Ile Asn Gly Pro Glu Ser  
 530 535 540  
 Val Leu Val Asn Thr Tyr Gln Trp Ile Ile Arg Asn Trp Glu Thr Val  
 545 550 555 560  
 Lys Ile Gln Trp Ser Gln Asn Pro Thr Met Leu Tyr Asn Lys Met Glu  
 565 570 575  
 Phe Glu Pro Phe Gln Ser Leu Val Pro Lys Ala Ile Arg Gly Gln Tyr  
 580 585 590  
 Ser Gly Phe Val Arg Thr Leu Phe Gln Gln Met Arg Asp Val Leu Gly  
 595 600 605  
 Thr Phe Asp Thr Thr Gln Ile Ile Lys Leu Leu Pro Phe Ala Ala Ala  
 610 615 620

Pro Pro Lys Gln Ser Arg Met Gln Phe Ser Ser Leu Thr Val Asn Val  
 625 630 635 640  
 Arg Gly Ser Gly Met Arg Ile Leu Val Arg Gly Asn Ser Pro Val Phe  
 645 650 655  
 Asn Tyr Asn Lys Thr Thr Lys Arg Leu Thr Ile Leu Gly Lys Asp Ala  
 660 665 670  
 Gly Thr Leu Thr Glu Asp Pro Asp Glu Gly Thr Ser Gly Val Glu Ser  
 675 680 685  
 Ala Val Leu Arg Gly Phe Leu Ile Leu Gly Lys Glu Asp Arg Arg Tyr  
 690 695 700  
 Gly Pro Ala Leu Ser Ile Asn Glu Leu Ser Asn Leu Ala Lys Gly Glu  
 705 710 715 720  
 Lys Ala Asn Val Leu Ile Gly Gln Gly Asp Val Val Leu Val Met Lys  
 725 730 735  
 Arg Lys Arg Asp Ser Ser Ile Leu Thr Asp Ser Gln Thr Ala Thr Lys  
 740 745 750  
 Arg Ile Arg Met Ala Ile Asn Xaa Cys Xaa Ile Val Xaa Lys Arg Pro  
 755 760 765  
 Cys Phe Tyr  
 770

<210> 10  
 <211> 757  
 <212> PRT  
 <213> Influenza virus A/Singapore/1/57/ca

<400> 10  
 Met Asp Val Asn Pro Thr Leu Leu Phe Leu Lys Val Pro Ala Gln Asn  
 1 5 10 15  
 Ala Ile Ser Thr Thr Phe Pro Tyr Thr Gly Asp Pro Pro Tyr Ser His  
 20 25 30  
 Gly Thr Gly Thr Gly Tyr Thr Met Asp Thr Val Asn Arg Thr His Gln  
 35 40 45  
 Tyr Ser Glu Lys Gly Lys Trp Thr Thr Asn Thr Glu Thr Gly Ala Pro  
 50 55 60

Gln	Leu	Asn	Pro	Ile	Asp	Gly	Pro	Leu	Pro	Glu	Asp	Asn	Glu	Pro	Ser	65	70	75	80
Gly	Tyr	Ala	Gln	Thr	Asp	Cys	Val	Leu	Glu	Ala	Met	Ala	Phe	Leu	Glu	85	90	95	
Glu	Ser	His	Pro	Gly	Ile	Phe	Glu	Asn	Ser	Cys	Leu	Glu	Thr	Met	Glu	100	105	110	
Val	Ile	Gln	Gln	Thr	Arg	Val	Asp	Lys	Leu	Thr	Gln	Gly	Arg	Gln	Thr	115	120	125	
Tyr	Asp	Trp	Thr	Leu	Asn	Arg	Asn	Gln	Pro	Ala	Ala	Thr	Ala	Leu	Ala	130	135	140	
Asn	Thr	Ile	Glu	Val	Phe	Arg	Ser	Asn	Gly	Leu	Thr	Ala	Asn	Glu	Ser	145	150	155	160
Gly	Arg	Leu	Ile	Asp	Phe	Leu	Lys	Asp	Val	Ile	Glu	Ser	Met	Asp	Lys	165	170	175	
Glu	Glu	Met	Glu	Ile	Thr	Thr	His	Phe	Gln	Arg	Lys	Arg	Arg	Val	Arg	180	185	190	
Asp	Asn	Met	Thr	Lys	Lys	Met	Val	Thr	Gln	Arg	Thr	Ile	Gly	Lys	Lys	195	200	205	
Lys	Gln	Arg	Leu	Asn	Lys	Arg	Ser	Tyr	Leu	Ile	Arg	Ala	Leu	Thr	Leu	210	215	220	
Asn	Thr	Met	Thr	Lys	Asp	Ala	Glu	Arg	Gly	Lys	Leu	Lys	Arg	Arg	Ala	225	230	235	240
Ile	Ala	Thr	Pro	Gly	Met	Gln	Ile	Arg	Gly	Phe	Val	Tyr	Phe	Val	Glu	245	250	255	
Thr	Leu	Ala	Arg	Ser	Ile	Cys	Glu	Lys	Leu	Glu	Gln	Ser	Gly	Leu	Pro	260	265	270	
Val	Gly	Gly	Asn	Glu	Lys	Lys	Ala	Lys	Leu	Ala	Asn	Val	Val	Arg	Lys	275	280	285	
Met	Met	Thr	Asn	Ser	Gln	Asp	Thr	Glu	Leu	Ser	Phe	Thr	Ile	Thr	Gly	290	295	300	
Asp	Asn	Thr	Lys	Trp	Asn	Glu	Asn	Gln	Asn	Pro	Arg	Met	Phe	Leu	Ala	305	310	315	320



Met Ile Thr Tyr Ile Thr Arg Asn Gln Pro Glu Trp Phe Arg Asn Val  
 325 330 335

Leu Ser Ile Ala Pro Ile Met Phe Ser Asn Lys Met Ala Arg Leu Gly  
 340 345 350

Lys Gly Tyr Met Phe Glu Ser Lys Ser Met Lys Leu Arg Thr Gln Ile  
 355 360 365

Pro Ala Glu Met Leu Ala Ser Ile Asp Leu Lys Tyr Phe Asn Glu Ser  
 370 375 380

Thr Arg Lys Lys Ile Glu Lys Ile Arg Pro Leu Leu Ile Asp Gly Thr  
 385 390 395 400

Val Ser Leu Ser Pro Gly Met Met Met Gly Met Phe Asn Met Leu Ser  
 405 410 415

Thr Val Ile Gly Val Ser Ile Leu Asn Leu Gly Gln Lys Lys Tyr Thr  
 420 425 430

Lys Thr Thr Tyr Trp Trp Asp Gly Leu Gln Ser Ser Asp Asp Phe Ala  
 435 440 445

Leu Ile Val Asn Ala Pro Asn His Glu Gly Ile Gln Ala Gly Val Asp  
 450 455 460

Arg Phe Tyr Arg Thr Cys Lys Leu Val Gly Ile Asn Met Ser Lys Lys  
 465 470 475 480

Lys Ser Tyr Ile Asn Arg Thr Gly Thr Phe Glu Phe Thr Ser Phe Phe  
 485 490 495

Tyr Arg Tyr Gly Phe Val Ala Asn Phe Ser Met Glu Leu Pro Ser Phe  
 500 505 510

Gly Val Ser Gly Ile Asn Glu Ser Ala Asp Met Ser Ile Gly Val Thr  
 515 520 525

Val Ile Lys Asn Asn Met Ile Asn Asn Asp Leu Gly Pro Ala Thr Ala  
 530 535 540

Gln Met Ala Leu Gln Leu Phe Ile Lys Asp Tyr Arg Tyr Thr Tyr Arg  
 545 550 555 560

Cys His Arg Gly Asp Thr Gln Ile Gln Thr Arg Arg Ser Phe Glu Leu  
 565 570 575

Lys Lys Leu Trp Glu Gln Thr Arg Ser Lys Ala Gly Leu Leu Val Ser  
 580 585 590  
 Asp Gly Gly Pro Asn Leu Tyr Asn Ile Arg Asn Leu His Ile Pro Glu  
 595 600 605  
 Val Cys Leu Lys Trp Glu Leu Met Asp Glu Asp Tyr Gln Gly Arg Leu  
 610 615 620  
 Cys Asn Pro Leu Asn Pro Phe Val Ser His Lys Glu Ile Glu Ser Val  
 625 630 635 640  
 Asn Asn Ala Val Val Met Pro Ala His Gly Pro Ala Lys Ser Met Glu  
 645 650 655  
 Tyr Asp Ala Val Ala Thr Thr His Ser Trp Ile Pro Lys Arg Asn Arg  
 660 665 670  
 Ser Ile Leu Asn Thr Ser Gln Arg Gly Ile Leu Glu Asp Glu Gln Met  
 675 680 685  
 Tyr Gln Lys Cys Cys Asn Leu Phe Glu Lys Phe Phe Pro Ser Ser Ser  
 690 695 700  
 Tyr Arg Arg Pro Val Gly Ile Ser Ser Met Val Glu Ala Met Val Ser  
 705 710 715 720  
 Arg Ala Arg Ile Asp Ala Arg Ile Asp Phe Glu Ser Gly Arg Ile Lys  
 725 730 735  
 Lys Glu Glu Phe Ala Glu Ile Met Lys Ile Cys Ser Thr Ile Glu Glu  
 740 745 750  
 Leu Arg Arg Gln Lys  
 755

&lt;210&gt; 11

&lt;211&gt; 716

&lt;212&gt; PRT

&lt;213&gt; Influenza virus A/Singapore/1/57/ca

&lt;400&gt; 11

Met Glu Asp Phe Val Arg Gln Cys Phe Asn Pro Met Ile Val Glu Leu  
 1 5 10 15

Ala Glu Arg Ala Met Lys Glu Tyr Gly Glu Asp Leu Lys Ile Glu Thr

	20		25		30	
Asn Lys Phe Ala Ala Ile Cys Thr His Leu Glu Val Cys Phe Met Tyr						
	35		40		45	
Ser Asp Phe His Phe Ile Asn Glu Gln Gly Glu Ser Ile Ile Val Glu						
	50		55		60	
Leu Asp Asp Pro Asn Ala Leu Leu Lys His Arg Phe Glu Ile Ile Glu						
	65		70		75	80
Gly Arg Asp Arg Thr Met Ala Trp Thr Val Val Asn Ser Ile Cys Asn						
		85		90		95
Thr Thr Gly Ala Glu Lys Pro Lys Phe Leu Pro Asp Leu Tyr Asp Tyr						
	100		105		110	
Lys Glu Asn Arg Phe Ile Glu Ile Gly Val Thr Arg Arg Glu Val His						
	115		120		125	
Ile Tyr Tyr Leu Glu Lys Ala Asn Lys Ile Lys Ser Glu Lys Thr His						
	130		135		140	
Ile His Ile Phe Ser Phe Thr Gly Glu Glu Met Ala Thr Lys Ala Asp						
	145		150		155	160
Tyr Thr Leu Asp Glu Glu Ser Arg Ala Arg Ile Lys Thr Arg Leu Phe						
		165		170		175
Thr Ile Arg Gln Glu Met Ala Ser Arg Gly Leu Trp Asp Ser Phe Arg						
	180		185		190	
Gln Ser Glu Arg Gly Glu Glu Thr Ile Glu Glu Arg Phe Glu Ile Thr						
	195		200		205	
Gly Thr Met Arg Arg Leu Ala Asp Gln Ser Leu Pro Pro Asn Phe Ser						
	210		215		220	
Cys Leu Glu Ile Phe Arg Ala Tyr Val Asp Gly Phe Glu Pro Asn Gly						
	225		230		235	240
Tyr Ile Glu Gly Lys Leu Ser Gln Met Ser Lys Glu Val Asn Ala Lys						
		245		250		255
Ile Glu Pro Phe Leu Lys Thr Thr Pro Arg Pro Ile Arg Leu Pro Asp						
	260		265		270	
Gly Pro Pro Cys Ser Gln Arg Ser Lys Phe Leu Leu Met Asp Ala Leu						

275	280	285
Lys Leu Ser Ile Glu Asp Pro Ser His Glu Gly Glu Gly Ile Pro Leu		
290	295	300
Tyr Asp Ala Ile Lys Cys Met Arg Thr Phe Phe Gly Trp Lys Glu Pro		
305	310	315
Tyr Val Val Lys Pro His Glu Lys Gly Ile Asn Pro Asn Tyr Leu Leu		
325	330	335
Ser Trp Lys Gln Val Leu Ala Glu Leu Gln Asp Ile Glu Asn Glu Glu		
340	345	350
Lys Ile Pro Arg Thr Lys Asn Met Lys Lys Thr Ser Gln Leu Lys Trp		
355	360	365
Ala Leu Gly Glu Asn Met Ala Pro Glu Lys Val Asp Phe Asp Asp Cys		
370	375	380
Arg Asp Ile Ser Asp Leu Lys Gln Tyr Asp Ser Asp Glu Pro Glu Leu		
385	390	395
Arg Ser Leu Ser Ser Trp Ile Gln Asn Glu Phe Asn Lys Ala Cys Glu		
405	410	415
Leu Thr Asn Ser Ile Trp Ile Glu Leu Asp Glu Ile Gly Glu Asp Val		
420	425	430
Ala Pro Ile Glu His Ile Ala Ser Met Arg Arg Asn Tyr Phe Thr Ala		
435	440	445
Glu Val Ser His Cys Arg Ala Thr Glu Tyr Ile Met Lys Gly Val Tyr		
450	455	460
Ile Asn Thr Ala Leu Leu Asn Ala Ser Cys Ala Ala Met Asp Asp Phe		
465	470	475
Gln Leu Ile Pro Met Ile Ser Lys Cys Arg Thr Lys Glu Gly Arg Arg		
485	490	495
Lys Thr Asn Leu Tyr Gly Phe Ile Val Lys Gly Arg Ser His Leu Arg		
500	505	510
Asn Asp Thr Asp Val Val Asn Phe Val Ser Met Glu Phe Ser Leu Thr		
515	520	525
Asp Pro Arg Leu Glu Pro His Lys Trp Glu Lys Tyr Cys Val Leu Glu		

530	535	540	
Ile Gly Asp Met Leu Leu Arg Ser Ala Ile Gly Gln Val Ser Arg Pro			
545	550	555	560
Met Phe Leu Tyr Val Arg Thr Asn Gly Thr Ser Lys Ile Lys Met Lys			
	565	570	575
Trp Gly Met Glu Met Arg Arg Cys Leu Leu Gln Ser Leu Gln Gln Ile			
	580	585	590
Glu Ser Met Ile Glu Ala Gln Ser Ser Val Lys Glu Lys Asp Met Thr			
	595	600	605
Lys Glu Phe Phe Glu Asn Lys Ser Glu Thr Trp Pro Ile Gly Glu Ser			
	610	615	620
Pro Lys Gly Val Glu Glu Gly Ser Ile Gly Lys Val Cys Arg Thr Leu			
	625	630	635
Leu Ala Lys Ser Val Phe Asn Ser Leu Tyr Ala Ser Pro Gln Leu Glu			
	645	650	655
Gly Phe Ser Ala Glu Ser Arg Lys Leu Leu Leu Val Val Gln Ala Leu			
	660	665	670
Arg Asp Asn Leu Glu Pro Gly Thr Phe Asp Leu Gly Gly Leu Tyr Glu			
	675	680	685
Ala Ile Glu Glu Cys Leu Ile Asn Asp Pro Trp Val Leu Leu Asn Ala			
	690	695	700
Ser Trp Phe Asn Ser Phe Leu Thr His Ala Leu Arg			
	705	710	715

&lt;210&gt; 12

&lt;211&gt; 562

&lt;212&gt; PRT

&lt;213&gt; Influenza virus A/Singapore/1/57/ca

&lt;400&gt; 12

Met Ala Ile Ile Tyr Leu Ile Leu Leu Phe Thr Ala Val Arg Gly Asp
1 5 10 15

Gln Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Lys Val Asp
20 25 30

Thr Ile Leu Glu Gln Asn Val Thr Val Thr His Ala Lys Asp Ile Leu  
 35 40 45

Glu Lys Thr His Asn Gly Lys Leu Cys Lys Leu Asn Gly Ile Pro Pro  
 50 55 60

Leu Glu Leu Gly Asp Cys Ser Ile Ala Gly Trp Leu Leu Gly Asn Pro  
 65 70 75 80

Glu Cys Asp Arg Leu Leu Ser Val Pro Glu Trp Ser Tyr Ile Met Glu  
 85 90 95

Lys Glu Asn Pro Arg Asp Gly Leu Cys Tyr Pro Gly Ser Phe Asn Asp  
 100 105 110

Tyr Glu Glu Leu Lys His Leu Leu Ser Ser Val Lys His Phe Glu Lys  
 115 120 125

Val Lys Ile Leu Pro Lys Asp Arg Trp Thr Gln His Thr Thr Thr Gly  
 130 135 140

Gly Ser Arg Ala Cys Ala Val Ser Gly Asn Pro Ser Phe Phe Arg Asn  
 145 150 155 160

Met Val Trp Leu Thr Lys Lys Glu Ser Asn Tyr Pro Val Ala Lys Gly  
 165 170 175

Ser Tyr Asn Asn Thr Ser Gly Glu Gln Met Leu Ile Ile Trp Gly Val  
 180 185 190

His His Pro Asn Asp Glu Thr Glu Gln Arg Thr Leu Tyr Gln Asn Val  
 195 200 205

Gly Thr Tyr Val Ser Val Gly Thr Ser Thr Leu Asn Lys Arg Ser Thr  
 210 215 220

Pro Asp Ile Ala Thr Arg Pro Lys Val Asn Gly Leu Gly Ser Arg Met  
 225 230 235 240

Glu Phe Ser Trp Thr Leu Leu Asp Met Trp Asp Thr Ile Asn Phe Glu  
 245 250 255

Ser Thr Gly Asn Leu Ile Ala Pro Glu Tyr Gly Phe Lys Ile Ser Lys  
 260 265 270

Arg Gly Asn Ser Gly Ile Met Lys Thr Glu Gly Thr Leu Glu Asn Cys  
 275 280 285

Glu Thr Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Thr Thr Leu Pro  
 290 295 300

Phe His Asn Val His Pro Leu Thr Ile Gly Glu Cys Pro Lys Tyr Val  
 305 310 315 320

Lys Ser Glu Lys Leu Val Leu Ala Thr Gly Pro Arg Asn Val Pro Gln  
 325 330 335

Ile Glu Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly  
 340 345 350

Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His His Ser Asn  
 355 360 365

Asp Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln Lys Ala  
 370 375 380

Phe Asp Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu Lys Met Asn  
 385 390 395 400

Thr Gln Phe Glu Ala Val Gly Lys Glu Phe Ser Asn Leu Glu Arg Arg  
 405 410 415

Leu Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp Val Trp  
 420 425 430

Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg Thr Leu  
 435 440 445

Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val Arg Met  
 450 455 460

Gln Leu Arg Asp Asn Val Lys Glu Leu Gly Asn Gly Cys Phe Glu Phe  
 465 470 475 480

Tyr His Lys Cys Asp Asp Glu Cys Met Asn Ser Val Lys Asn Gly Thr  
 485 490 495

Tyr Asp Tyr Pro Lys Tyr Glu Glu Glu Ser Lys Leu Asn Arg Asn Glu  
 500 505 510

Ile Lys Gly Val Lys Leu Ser Ser Met Gly Val Tyr Gln Ile Leu Ala  
 515 520 525

Ile Tyr Ala Thr Val Ala Gly Ser Leu Ser Leu Ala Ile Met Met Ala  
 530 535 540

Gly Ile Ser Phe Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile  
 545 550 555 560

Cys Ile

<210> 13

<211> 506

<212> PRT

<213> Influenza virus A/Singapore/1/57/ca

<400> 13

Met Ala Ser Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Asp  
 1 5 10 15

Gly Glu Arg Gln Asn Ala Thr Glu Ile Arg Ala Ser Val Gly Lys Met  
 20 25 30

Ile Asp Gly Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys  
 35 40 45

Leu Ser Asp Tyr Glu Gly Arg Leu Ile Gln Asn Ser Leu Thr Ile Glu  
 50 55 60

Arg Met Val Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu  
 65 70 75 80

Glu His Pro Ser Ala Gly Lys Asp Pro Lys Lys Thr Gly Gly Pro Ile  
 85 90 95

Tyr Lys Arg Val Asn Gly Lys Trp Met Arg Glu Leu Val Leu Tyr Asp  
 100 105 110

Lys Glu Glu Ile Arg Arg Ile Trp Arg Gln Ala Asn Asn Gly Asp Asp  
 115 120 125

Ala Thr Ala Gly Leu Thr His Met Met Ile Trp His Ser Asn Leu Asn  
 130 135 140

Asp Thr Thr Tyr Gln Arg Thr Arg Ala Leu Val Arg Thr Gly Met Asp  
 145 150 155 160

Pro Arg Met Cys Ser Leu Met Gln Gly Ser Thr Leu Pro Arg Arg Ser  
 165 170 175

Gly Ala Ala Gly Ala Ala Val Lys Gly Val Gly Thr Met Val Met Glu  
 180 185 190



Leu Ile Arg Met Ile Lys Arg Gly Ile Asn Asp Arg Asn Phe Trp Arg  
 195 200 205

Gly Glu Asn Gly Arg Lys Thr Arg Ile Ala Tyr Glu Arg Met Cys Asn  
 210 215 220

Ile Leu Lys Gly Lys Phe Gln Thr Ala Ala Gln Arg Ala Met Met Asp  
 225 230 235 240

Gln Val Arg Glu Ser Arg Asn Pro Gly Asn Ala Glu Ile Glu Asp Leu  
 245 250 255

Ile Phe Leu Ala Arg Ser Ala Leu Ile Leu Arg Gly Ser Val Ala His  
 260 265 270

Lys Ser Cys Leu Pro Ala Cys Val Tyr Gly Thr Ala Val Ala Ser Gly  
 275 280 285

Tyr Asp Phe Glu Lys Glu Gly Tyr Ser Leu Val Gly Ile Asp Pro Phe  
 290 295 300

Lys Leu Leu Gln Asn Ser Gln Val Tyr Ser Leu Ile Arg Pro Asn Glu  
 305 310 315 320

Asn Pro Ala His Lys Ser Gln Leu Val Trp Met Ala Cys Asn Ser Ala  
 325 330 335

Ala Phe Glu Asp Leu Arg Val Ser Ser Phe Ile Arg Gly Thr Lys Val  
 340 345 350

Ile Pro Arg Gly Lys Leu Ser Thr Arg Gly Val Gln Ile Ala Ser Asn  
 355 360 365

Glu Asn Met Asp Thr Met Glu Ser Ser Thr Leu Glu Leu Arg Ser Arg  
 370 375 380

Tyr Trp Ala Ile Arg Thr Arg Ser Gly Gly Asn Thr Asn Gln Gln Arg  
 385 390 395 400

Ala Ser Ala Gly Gln Ile Ser Val Gln Pro Thr Phe Ser Val Gln Arg  
 405 410 415

Asn Leu Pro Phe Asp Lys Thr Thr Ile Met Ala Ala Phe Thr Gly Asn  
 420 425 430

Ala Glu Gly Arg Thr Ser Asp Met Arg Ala Glu Ile Ile Arg Met Met  
 435 440 445

Glu Gly Ala Lys Pro Glu Glu Val Ser Phe Gln Gly Arg Gly Val Phe  
 450 455 460

Glu Leu Ser Asp Glu Lys Ala Thr Asn Pro Ile Val Pro Ser Phe Asp  
 465 470 475 480

Met Ser Asn Glu Gly Ser Tyr Phe Phe Gly Asp Asn Ala Glu Glu Tyr  
 485 490 495

Asp Asn Xaa Gly Lys Ile Pro Leu Phe Leu  
 500 505

<210> 14

<211> 469

<212> PRT

<213> Influenza virus A/Singapore/1/57/ca

<400> 14

Met Asn Pro Asn Gln Lys Ile Ile Thr Ile Gly Ser Val Ser Leu Thr  
 1 5 10 15

Ile Ala Thr Val Cys Phe Leu Met Gln Ile Ala Ile Leu Ala Thr Thr  
 20 25 30

Val Thr Leu His Phe Lys Gln His Glu Cys Asp Ser Pro Ala Ser Asn  
 35 40 45

Gln Val Met Pro Cys Glu Pro Ile Ile Ile Glu Arg Asn Ile Thr Glu  
 50 55 60

Ile Val Tyr Leu Asn Asn Thr Thr Ile Glu Lys Glu Ile Cys Pro Glu  
 65 70 75 80

Val Val Glu Tyr Arg Asn Trp Ser Lys Pro Gln Cys Gln Ile Thr Gly  
 85 90 95

Phe Ala Pro Phe Ser Lys Asp Asn Ser Ile Arg Leu Ser Ala Gly Gly  
 100 105 110

Asp Ile Trp Val Thr Arg Glu Pro Tyr Val Ser Cys Asp Pro Gly Lys  
 115 120 125

Cys Tyr Gln Phe Ala Leu Gly Gln Gly Thr Thr Leu Tyr Asn Lys His  
 130 135 140

Ser Asn Gly Thr Ile His Asp Arg Ile Pro His Arg Thr Leu Leu Met

145                      150                      155                      160  
 Asn Glu Leu Gly Val Pro Phe His Leu Gly Thr Lys Gln Val Cys Val  
                                  165                      170                      175  
 Ala Trp Ser Ser Ser Ser Cys His Asp Gly Lys Ala Trp Leu His Val  
                                  180                      185                      190  
 Cys Val Thr Gly Asp Asp Arg Asn Ala Thr Ala Ser Phe Ile Tyr Asp  
                                  195                      200                      205  
 Gly Arg Leu Val Asp Ser Ile Gly Ser Trp Ser Gln Asn Ile Leu Arg  
                                  210                      215                      220  
 Thr Gln Glu Ser Glu Cys Val Cys Ile Asn Gly Thr Cys Thr Val Val  
 225                      230                      235                      240  
 Met Thr Asp Gly Ser Ala Ser Gly Arg Ala Asp Thr Arg Ile Leu Phe  
                                  245                      250                      255  
 Ile Lys Glu Gly Lys Ile Val Arg Ile Ser Pro Leu Ser Gly Ser Ala  
                                  260                      265                      270  
 Gln His Ile Glu Glu Cys Ser Cys Tyr Pro Arg Tyr Pro Asp Val Arg  
                                  275                      280                      285  
 Cys Ile Cys Arg Asp Asn Trp Lys Gly Ser Asn Arg Pro Val Ile Asp  
                                  290                      295                      300  
 Ile Asn Met Glu Asp Tyr Ser Ile Asp Ser Ser Tyr Val Cys Ser Gly  
 305                      310                      315                      320  
 Leu Val Gly Asp Thr Pro Arg Asn Asp Asp Ser Ser Ser Asn Ser Asn  
                                  325                      330                      335  
 Cys Arg Asp Pro Asn Asn Glu Arg Gly Asn Pro Gly Val Lys Gly Trp  
                                  340                      345                      350  
 Ala Phe Asp Asn Gly Asp Asp Val Trp Met Gly Arg Thr Ile Asn Lys  
                                  355                      360                      365  
 Asp Ser Arg Ser Gly Tyr Glu Thr Phe Lys Val Ile Gly Gly Trp Ser  
                                  370                      375                      380  
 Thr Pro Asn Ser Lys Ser Gln Val Asn Arg Gln Val Ile Val Asp Asn  
 385                      390                      395                      400  
 Asn Asn Trp Ser Gly Tyr Ser Gly Ile Phe Ser Val Glu Gly Lys Ser

405                      410                      415  
 Cys Ile Asn Arg Cys Phe Tyr Val Glu Leu Ile Arg Gly Arg Pro Gln  
                     420                      425                      430  
 Glu Thr Arg Val Trp Trp Thr Ser Asn Ser Ile Val Val Phe Cys Gly  
                     435                      440                      445  
 Thr Ser Gly Thr Tyr Gly Thr Gly Ser Trp Pro Asp Gly Ala Asn Ile  
                     450                      455                      460  
 Asn Phe Met Pro Ile  
 465

<210> 15  
 <211> 252  
 <212> PRT  
 <213> Influenza virus A/Singapore/1/57/ca

<400> 15  
 Met Ser Leu Leu Thr Glu Val Glu Thr Tyr Val Leu Ser Ile Val Pro  
   1                      5                      10                      15  
 Ser Gly Pro Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asp Val Phe  
                     20                      25                      30  
 Ala Gly Lys Asn Thr Asp Leu Glu Ala Leu Met Glu Trp Leu Lys Thr  
                     35                      40                      45  
 Arg Pro Ile Leu Ser Pro Leu Thr Lys Gly Ile Leu Gly Phe Val Phe  
                     50                      55                      60  
 Thr Leu Thr Val Pro Ser Glu Arg Gly Leu Gln Arg Arg Arg Phe Val  
                     65                      70                      75                      80  
 Gln Asn Ala Leu Asn Gly Asn Gly Asp Pro Asn Asn Met Asp Arg Ala  
                     85                      90                      95  
 Val Lys Leu Tyr Lys Lys Leu Lys Arg Glu Ile Thr Phe His Gly Ala  
                     100                      105                      110  
 Lys Glu Ile Ala Leu Ser Tyr Ser Ala Gly Ala Leu Ala Ser Cys Met  
                     115                      120                      125  
 Gly Leu Ile Tyr Asn Arg Met Gly Ala Val Thr Thr Glu Val Ala Phe  
                     130                      135                      140

Gly Leu Val Cys Ala Thr Cys Glu Gln Ile Ala Asp Ser His His Arg  
 145 150 155 160

Ser His Arg Gln Met Val Thr Thr Thr Asn Pro Leu Ile Arg His Glu  
 165 170 175

Asn Arg Met Val Leu Ala Ser Thr Thr Ala Lys Ala Met Glu Gln Met  
 180 185 190

Ala Gly Ser Ser Glu Gln Ala Ala Glu Ala Met Glu Val Ala Ser Gln  
 195 200 205

Ala Arg Gln Met Val Gln Ala Met Arg Ala Ile Gly Thr His Pro Ser  
 210 215 220

Ser Ser Ala Gly Leu Lys Asp Asp Leu Leu Glu Asn Leu Gln Ala Tyr  
 225 230 235 240

Gln Lys Arg Met Gly Val Gln Met Gln Arg Phe Lys  
 245 250

<210> 16

<211> 97

<212> PRT

<213> Influenza virus A/Singapore/1/57/ca

<400> 16

Met Ser Leu Leu Thr Glu Val Glu Thr Pro Ile Arg Asn Glu Trp Gly  
 1 5 10 15

Cys Arg Cys Asn Asp Ser Ser Asp Pro Leu Val Val Ala Ala Ser Ile  
 20 25 30

Ile Gly Ile Leu His Leu Ile Leu Trp Ile Leu Asp Arg Leu Phe Phe  
 35 40 45

Lys Cys Ile Tyr Arg Phe Phe Lys His Gly Leu Lys Arg Gly Pro Ser  
 50 55 60

Thr Glu Gly Val Pro Glu Ser Met Arg Glu Glu Tyr Arg Lys Glu Gln  
 65 70 75 80

Gln Ser Ala Val Asp Ala Asp Asp Ser His Phe Val Ser Ile Glu Leu  
 85 90 95

Glu

&lt;210&gt; 17

&lt;211&gt; 237

&lt;212&gt; PRT

&lt;213&gt; Influenza virus A/Singapore/1/57/ca

&lt;400&gt; 17

Met Asp Pro Asn Thr Val Ser Ser Phe Gln Val Asp Cys Phe Leu Trp  
 1 5 10 15

His Val Arg Lys Gln Val Ala Asp Gln Glu Leu Gly Asp Ala Pro Phe  
 20 25 30

Leu Asp Arg Leu Arg Arg Asp Gln Lys Ser Leu Arg Gly Arg Gly Ser  
 35 40 45

Thr Leu Gly Leu Asn Ile Glu Thr Ala Thr Arg Val Gly Lys Gln Ile  
 50 55 60

Val Glu Arg Ile Leu Lys Glu Glu Ser Asp Glu Ala Leu Lys Met Thr  
 65 70 75 80

Met Ala Ser Ala Pro Ala Ser Arg Tyr Leu Thr Asp Met Thr Ile Glu  
 85 90 95

Glu Met Ser Arg Asp Trp Phe Met Leu Met Pro Lys Gln Lys Val Ser  
 100 105 110

Gly Pro Leu Cys Ile Arg Met Asp Gln Ala Ile Met Asp Lys Asn Ile  
 115 120 125

Ile Leu Lys Ala Asn Phe Ser Val Ile Phe Asp Arg Leu Glu Thr Leu  
 130 135 140

Ile Leu Leu Arg Ala Phe Thr Glu Glu Gly Ala Ile Val Gly Glu Ile  
 145 150 155 160

Ser Pro Leu Pro Ser Leu Pro Gly His Thr Asn Glu Asp Val Lys Asn  
 165 170 175

Ala Ile Gly Val Leu Ile Gly Gly Leu Glu Trp Asn Asp Asn Thr Val  
 180 185 190

Arg Val Ser Lys Thr Leu Gln Arg Phe Ala Trp Arg Asn Ser Asn Glu  
 195 200 205

Asn Gly Arg Pro Pro Leu Thr Pro Lys Gln Lys Arg Lys Met Ala Arg

210                                      215                                      220  
 Thr Ile Arg Ser Lys Val Arg Arg Asn Lys Met Ala Asp  
 225                                      230                                      235  
  
 <210> 18  
 <211> 121  
 <212> PRT  
 <213> Influenza virus A/Singapore/1/57/ca  
  
 <400> 18  
 Met Asp Pro Asn Thr Val Ser Ser Phe Gln Asp Ile Leu Met Arg Met  
   1                                      5                                      10                                      15  
  
 Ser Lys Met Gln Leu Gly Ser Ser Ser Glu Asp Leu Asn Gly Met Ile  
                                     20                                      25                                      30  
  
 Thr Gln Phe Glu Ser Leu Lys Leu Tyr Arg Asp Ser Leu Gly Glu Thr  
                                     35                                      40                                      45  
  
 Val Met Arg Met Gly Asp Leu His Ser Leu Gln Asn Arg Asn Gly Lys  
   50                                      55                                      60  
  
 Trp Arg Glu Gln Leu Gly Gln Lys Phe Glu Glu Ile Arg Trp Leu Ile  
   65                                      70                                      75                                      80  
  
 Glu Glu Val Arg His Lys Leu Lys Ile Thr Glu Asn Ser Phe Glu Gln  
                                     85                                      90                                      95  
  
 Ile Thr Phe Met Gln Ala Leu Gln Leu Leu Phe Glu Val Glu Gln Glu  
                                     100                                      105                                      110  
  
 Ile Arg Thr Phe Ser Phe Gln Leu Ile  
                                     115                                      120

<210> 19  
 <211> 2396  
 <212> DNA  
 <213> Influenza B/Vienna/1/99/ca

<400> 19  
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 taagaaaatt caatacatca agaattgaaa agaacccttc attaaggatg aagtgggcaa 180  
 tgtgttctaa ttttccttg gctttgacca agggtgacat ggcaaacaga atccccttgg 240  
 aatacaaggg aatacaactt aaaacaaatg ctgaagacat aggaacccaa ggccaaatgt 300

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gctcaatagc agcagttacc tgggtggaata catatggacc aataggagat actgaagggtt 360
tcgaaaaagt ctacgaaagc ttttttctca gaaagatgag acttgacaat gccacttggg 420
gccgaataac ttttggccca gttgaaagag taagaaaaag ggtactgcta aacctctca 480
ccaaggaaat gcctccagat gaagcaagta atgtgataat ggaaatattg ttccctaagg 540
aagcaggaat accaagagaa tctacttgga tacatagga actgataaaa gaaaaaagag 600
aaaaattgaa aggaacgatg ataactccca ttgtactggc atacatgctt gagagggagt 660
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aaggaattaa gagacaaaga atgacagttg agtccatggg gtgggccttg agctaataa 2340
aatttatcca ttaattcaat gaatgcaatt gagtgaaaaa tgctcgtgtt tctcat 2396

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&lt;210&gt; 20

&lt;211&gt; 2369

&lt;212&gt; DNA

&lt;213&gt; Influenza B/Vienna/1/99/ca

&lt;400&gt; 20

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agcagaagcg gagcctttta gatgaatata aatccttatt ttctcttcat agatgtaccc 60
atacaggcag caatttcaac aacattccca tacaccggtg tccccctta ttccatgga 120
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aaacagtatg tttctgacat cacaggatgt acaatggtag atccaacaaa tggaccatta 240
cccgaagaca atgagccaag tgcttatgca caattagatt gcgttctgga ggctttggat 300
agaatggatg aggaacatcc aggtctgttt caagcagcct cacagaatgc catggaggca 360

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ctaattgtca caactgtaga caaattaacc caggggagac agactttcga ttggacagta 420
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gatttgaatg gagctgacaa ggggtgattg gtaccctttt gccagatat cattgattca 540
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gctccgaaga tgtccatggg gttattggtc atcattggat acatgtgata aacaaatgat 2340
taaaatgaaa aaaggctcgt gtttctact 2369

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&lt;210&gt; 21

&lt;211&gt; 2305

&lt;212&gt; DNA

&lt;213&gt; Influenza B/Vienna/1/99/ca

&lt;400&gt; 21

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agcagaagcg gtgcgtttga tttgtcataa tggatacttt tattacaaga aacttccaga 60
ctacaataat acaaaaggcc aaaaacacaa tggcagaatt tagtgaagat cctgaattac 120
aaccagcaat gctattcaac atctgcgtcc atctagagggt ttgctatgta ataagtgaca 180
tgaattttct tgacgaagaa ggaaaagcat atacagcatt agaaggacaa gggaaagAAC 240
aaaatttgag accacaatat gaagtaattg agggaatgcc aagaaccata gcatggatgg 300
tccaaagatc cttagctcaa gagcatggaa tagagactcc caagtatctg gctgatttgt 360

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ttgattataa aaccaagaga tttatagaag ttggaataac aaaaggattg gctgatgatt 420
acttttggaa aaagaaagaa aagctgggaa atagcatgga actgatgata ttcagctaca 480
atcaagacta ttcgttaagt aatgaatcct cattggatga ggaagggaaa gggagagtgc 540
taagcagact cacagaactt caggctgaat taagtctgaa aaacctatgg caagtcttca 600
taggagaaga agatgttgaa aagggaattg actttaaact tggacaaaca atatctagac 660
taagggatat atctgttcca gctggtttct ccaattttga aggaatgagg agctacatag 720
acaatataga cccgaaagga gcaatagaga gaaatctagc aaggatgtct cccttagtat 780
cagtcacacc taaaaagttg aaatgggagg acctaagacc aatagggcct cacatttaca 840
accatgagct accagaagtt ccatataatg cctttcttct aatgtctgat gaactggggc 900
tggccaatat gactgaggga aagtccaaaa aaccgaagac attagccaaa gaatgtctag 960
aaaagtactc aacactacgg gatcaaaactg acccaatatt aataatgaaa agcgaaaaag 1020
ctaacgaaaa tttcctatgg aagctttgga gagactgtgt aaatacaata agtaatgagg 1080
aaatgagtaa cgagttacag aaaaccaatt atgccaaagt ggccacaggg gatggattaa 1140
cataccagaa aataatgaaa gaagtagcaa tagatgacga aacaatgtgc caagaagagc 1200
ctaaaatccc taacaaatgt agagtggctg cttgggttca aacagagatg aatctattga 1260
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cctctacagt tatgatgaag tatgtgcttt ttcacacttc attgttgaat gaaagcaatg 1440
ccagcatggg aaaatacaaa gtaataccaa taaccaatag agtagtaaat gaaaaaggag 1500
aaagtttctga catgctctat ggtctggcgg ttaaaggaca atctcatctg aggggagata 1560
ctgatgttgt aacagttgta actttcgaat ttagtagtac agacccaga gtggactcag 1620
gaaagtggcc aaaatatact gtgttttagga ttggctccct atttgtgagt gggagggaaa 1680
agtctgtgta cctatattgc cgagtgaatg gcacaaataa gatccaaatg aaatggggaa 1740
tggagctag aagatgtctg cttcaatcaa tgcaacaaat ggaagcaatt gttgaacaag 1800
aatcatcgat acaaggatat gacatgacca aagcttgttt caaggagagac agagttaaata 1860
gccccaaaac tttcagtatt ggaactcaag aaggaaaact agtaaaagga tcctttggaa 1920
aagcactaag agtaatatatt actaaatggt tgatgcacta tgtatttggga aatgcccaat 1980
tggaggggtt tagtgccgag tctaggagac ttctactgtt gattcaagca ttaaaggaca 2040
gaaagggccc ttgggtgttc gacttagagg gaatgtattc tggaatagaa gaatgtatta 2100
gtaacaacc cttgggttaata cagagtgcac actgggttcaa tgaatggttg ggctttgaaa 2160
aggaggggag taaagtatta gaatcagtag atgaaataat ggatgaataa aaggacatag 2220
tactcaatatt agtactatatt tgttcattat gtatctaaac atccaataaa aaggacaaag 2280
aattaaaaat gcacgtgttt ctact 2305

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&lt;210&gt; 22

&lt;211&gt; 1882

&lt;212&gt; DNA

&lt;213&gt; Influenza B/Vienna/1/99/ca

&lt;400&gt; 22

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agcagaagca gagcattttc taatatccac aaaatgaagg caataattgt actactcatg 60
gtagtaacat ccaatgcaga tcgaatctgc actgggataa catcgtcaaa ctcacctcat 120
gtgggtcaaaa cagctactca aggggaggtc aatgtgactg gtgcgatacc actgacaaca 180
acaccaacaa aatctcattt tgcaaatctc aaaggaacaa agaccagagg gaaactatgc 240
ccaacctgtc tcaactgcac agatctggat gtggccttgg gcagaccaat gtgtgtgggg 300
atcacacctt cggcaaaaagc ttcaatactc cacgaagtca gacctgttac atccggatgc 360
tttcctataa tgcacgacag aacaaaaatc agacagctac ccaatcttct cagaggatat 420
gaaaaaatca gattatcaac ccaaacggtt atcaacacag aaaaggcacc aggaggaccc 480

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tacagacttg gaacttcagg atcttgcctt aacgctacca gtaaaagcgg atttttcgca 540
acaatggctt gggctgtccc aagggacaac aacaaaacag caacgaatcc actaacagta 600
gaagtaccac acatctgtac aaaagaagaa gaccaaatta ctgtttgggg gttccattct 660
gataacaaaa cccaaatgaa aaacctctat ggagactcaa atcctcaaaa gttcacctca 720
tctgctaata ggataaccac acattatgtt tctcagattg gcggcttccc ggaccaaaca 780
gaagacggag ggctaccaca aagcggcaga attggttgtt attacatggt gcaaaaacct 840
gggaaaacag gaacaattgt ctatcaaaga gggattttgt tgcctcaaaa ggtgtggtgc 900
gcgagtggca ggagcaaaagt aataaaaggg tccttgcctt taattggtga agcagattgc 960
cttcacgaaa aatacggtag attaaacaaa agcaagcctt actacacagg agaacatgca 1020
aaagccatag gaaattgccc aatatgggtg aaaacacctt tgaagcttgc caatggaacc 1080
aaatatagac ctctgcgaaa actattaaag gaaaggggtt tcttcggagc tattgctggt 1140
ttcttagaag gaggatggga aggaatgatt gcaggttggc acggatacac atctcacgga 1200
gcacatggag tggcagtggc agcagacctt aagagtacgc aagaagccat aaacaagata 1260
acaaaaatc tcaattcttt gagtgagcta gaagtaaata accttcaaag actaagtgg 1320
gccatggatg aactccataa cgaaatactc gagctggatg agaaagtgga tgatctcaga 1380
gctgacacaa taagctcaca aatagaactt gcagtcttgc tttccaacga aggaataata 1440
aacagtgaag atgagcatct attggcactt gagagaaaac taaagaaaat gctgggtccc 1500
tctgctgtag acatagggaa tggatgcttc gaaaccaaac acaagtcaa ccagacctgc 1560
ttagacagga tagctgctgg cacctttaat gcagaagaat tttctcttcc cacttttgat 1620
tactgaaca ttactgctgc atctttaaat gatgatggat tggataacca tactatactg 1680
ctctactact caactgctgc ttctagtgtt gctgtaacat tgatgatagc tatttttatt 1740
gtttatatga tctccagaga caatgtttct tgctccatct gtctataagg aaaattaagc 1800
cctgtatttt cttttattgt ggtgcttgtt tgcttggtat cattacaaag aaacgttatt 1860
gaaaaatgct cttgttacta ct 1882

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&lt;210&gt; 23

&lt;211&gt; 1844

&lt;212&gt; DNA

&lt;213&gt; Influenza B/Vienna/1/99/ca

&lt;400&gt; 23

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agcagaagca cagcattttc ttgtgaactt caagtaccag taaaagaact gaaaatcaaa 60
atgtccaaca tggatattga cggatcaac actgggacaa ttgacaaaac accggaagaa 120
ataacttttg gaaccagtgg gacaaccaga ccaatcatca gaccagcaac ccttgcccca 180
ccaagcaaca aacgaaccgg taaccatcc ccggaagag caaccacaag cagtgaagct 240
gatgtcggga ggaaaacca aaagaaacag acccgacag agataaagaa gagcgtctac 300
aacatggtag tgaaactggg cgaattctac aaccagatga tggtaaagc tggactcaac 360
gatgacatgg agagaaacct aatccaaaat gcgcatgctg tggaaagaat tctattggct 420
gccactgatg acaagaaaac tgaattccag aagaaaaaga ataccagaga tgtcaaagaa 480
gggaaagaag aaatagatca caacaaaaca ggaggcacct tttacaagat ggtaagagat 540
gataaaacca tctacttcag ccctataaga attacctttt taaaagaaga ggtgaaaaca 600
atgtacaaaa ccaccatggg gagtgatggc ttcagtggac taaatcacat aatgattggg 660
cattcacaga tgaatgatgt ctgtttccaa agatcaaagg cactaaaaag agttggactt 720
gacccttcat taatcagtac ctttgcggga agcacaatcc ccagaagatc aggtgcaact 780
ggtgttgcaa tcaaaggagg tggaaacttta gtggctgaag ccattcgatt tataggaaga 840
gcaatggcag acagagggtt attgagagac atcaaagcca agactgccta tgaaaagatt 900
cttctgaatc taaaaaaca atgctctgcg cccaacaaa aggtctctagt tgatcaagtg 960
atcggaaagta gaaatccagg gattgcagac attgaagatc taaccctgct tgctcgtagt 1020

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atggtcggtg ttaggcctc tgtggcgagc aaagtgggtg ttcccataag catttacgcc 1080
aaaatacctc aactagggtt caatgttgaa gagtactcta tggttgggta cgaagccatg 1140
gctctttaca atatggcaac acctgtttcc atattaagaa tgggagatga tgcaaaagat 1200
aagtcgcaat tattcttcat gtcttgcttc ggagctgcct atgaagacct gagagttttg 1260
tctgcattaa caggcacaga attcaagcct agatcagcat taaaatgcaa gggtttccat 1320
gttccagcaa aggaacaggt ggaaggaatg ggggcagctc tgatgtccat caagctccag 1380
ttttgggctc caatgaccag atctgggggg aacgaagtag gtggagacgg agggctctggc 1440
caaataagct gcagcccagt gtttgcaagt gaaagacctt ttgctctaag caagcaagct 1500
gtaagaagaa tgctgtcaat gaattattgag ggacgtgatg cagatgtcaa aggaaatcta 1560
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accatcccca atttcttctt tgggagggac acagcagagg attatgatga cctcgattat 1740
taaagcaaca aaatagacac tatgactgtg attgtttcaa tacgtttgga atgtgggtgt 1800
ttattcttat taaaataaat ataaaaaatg ctgttgtttc tact 1844

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&lt;210&gt; 24

&lt;211&gt; 1557

&lt;212&gt; DNA

&lt;213&gt; Influenza B/Vienna/1/99/ca

&lt;400&gt; 24

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agcagaagca gagcatcttc tcaaaactga ggcaaatagg caaaaaatga acaatgctac 60
cttcaactat acaaacgtta accctattcc tcacatcagg gggagtgtta ttatcactat 120
atgtgtcagc ttcaactgtca tacttattat attcggatat attgctaaaa ttttcaccaa 180
cagaaataac tgcaccaaca atgccattgg attgtgcaaa cgcatcaaat gttcaggctg 240
tgaaccgttc tgcaacaaaa ggggtgacac ttcttctccc agaaccggag tggacatacc 300
cgcgtttatc ttgccogggc tcaacctttc agaaagcact cctaattagc cctcatagat 360
tcggagaaac caaaggaaac tcagctccct tgataataag ggaacctttt attgcttgtg 420
gaccaaagga atgcaaacac tttgctctaa ccattatgc agcccaacca gggggatact 480
acaatggaac aagagaagac agaacaagc tgaggcatct aatttcagtc aaattgggca 540
aatcccaac agtagaaaac tccattttcc acatggcagc atggagcggg tccgcatgcc 600
atgatggtaa agaattggaca tatatcggag ttgatggccc tgacagtaat gcattgtctca 660
aaataaaaata tggagaagca tatactgaca cataccattc ctatgcaaac aacatcctaa 720
gaacacaaga aagtgcctgc aattgcatcg ggggaaattg ttatcttatg ataactgatg 780
gctcagcttc aggtattagt gaatgcagat ttcttaagat tcaagagggc cgaataataa 840
aagaaatatt tccaacagga agagtagaac atactgaaga atgcacatgc ggatttgoca 900
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tcaaattaaa tgtggagact gatacagcag aaataagatt gatgtgcaca gagacttact 1020
tggacacccc cagaccagat gatggaagca taacaggggc ttgtgaatct aatggggata 1080
aagggagtgg aggcattcaag ggaggatttg ttcatcaaag aatggcatcc aagactggaa 1140
ggtggtactc tcgaacaatg tctaaaacta aaaggatggg gatgggactg tatgtcaagt 1200
atgatggaga cccatggact gacagtgatg cccttgctct tagtggagta atggtttcaa 1260
tggaagaacc tggttggtac tcctttggct tcgaaataaa agataagaaa tgtgatgtcc 1320
cctgtattgg gatagagatg gtacatgatg gtggaagga gacttggcac tcagcagcaa 1380
cagccattta ctgtttaatg ggctcaggac aactgctatg ggacactgtc acagggtgta 1440
atatggctct gtaatggagg aatggttgag tctgttctaa accctttgtt cctattttgt 1500
ttgaacaatt gtccttactg aacttaattg tttctgaaaa atgctcttgt tactact 1557

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<210> 25  
 <211> 1190  
 <212> DNA  
 <213> Influenza B/Vienna/1/99/ca

<400> 25  
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 ggtgggaaag aatttgacct agactctgcc ttggaatgga taaaaaacia aagatgctta 180  
 actgatatac aaaaagcact aattgggtgcc tctatctgct ttttaaaacc caaagaccag 240  
 gaaagaaaaa gaagattcat cacagagccc ctatcaggaa tgggaacaac agcaacaaaa 300  
 aagaaaggcc tgattctagc tgagagaaaa atgagaagat gtgtgagctt tcatgaagca 360  
 tttgaaatag cagaaggcca tgaaagctca gcgctactat attgtctcat ggtcatgtac 420  
 ctgaatcctg gaaattattc aatgcaagta aaactaggaa cgctctgtgc tttgtgcgag 480  
 aaacaagcat cacattcaca cagggtctcat agcagagcag cgagatcttc agtgcccgga 540  
 gtgagacgag aaatgcagat ggtctcagct atgaacacag caaaaacaat gaatggaatg 600  
 ggaaaaggag aagacgtcca aaaactggca gaagagctgc aaagcaacat tggagtactg 660  
 agatctcttg gggcaagtca aaagaatggg gaaggaattg caaaggatgt aatggaagtg 720  
 ctaaagcaga gctctatggg aaattcagct cttgtgaaga aatatctata atgctcgaa 780  
 catttcagat tctttcaatt tgttctttta tcttatcagc tctccatttc gtggcttggg 840  
 caatagggca tttgaatcaa ataaaaagag gagtaaacat gaaaatacga ataaaaagtc 900  
 caaacaaga gacaataaac agagaggtat caattttgag acacagttac caaaaagaaa 960  
 tccaggccaa agaaacaatg aaggaagtac tctctgacaa catggaggta ttgggtgacc 1020  
 acatagtaat tgaggggctt tctgccgaag agataataaa aatgggtgaa acagttttgg 1080  
 agatagaaga attgcattaa attcaatttt tactgtatct cttactatgc atttaagcaa 1140  
 attgtaatca atgtcagcaa ataaactgga aaaagtgcgt tgtttctact 1190

<210> 26  
 <211> 1097  
 <212> DNA  
 <213> Influenza B/Vienna/1/99/ca

<400> 26  
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 cacaacacaa attgaggtgg gtccgggagc aaccaatgcc accataaact ttgaaacagg 120  
 aattctggag tgctatgaaa ggctttcatg gcaaagagcc cttgactacc ctggtcaaga 180  
 ccgcctaaac agactaaaga gaaaattaga gtcaagaata aagactcaca acaaaagtga 240  
 gcctgaaagt aaaaggatgt ctcttgaaga gaggaagca attggagtaa aaatgatgaa 300  
 agtactccta tttatgaatc catctgctgg aattgaaggg tttgagccat actatatgaa 360  
 aagttcctca aatagcaact gtccgaaata caattggacc gattaccctt caacaccagg 420  
 gaggtgcctt gatgacatag aagaagaacc agaggatggt gatggcccaa ctgaaatagt 480  
 attaagggac atgaacaaca aagatgcaag gcaaaagata aaagaggag taaacactca 540  
 gaaagaaggg aagttccgtt tgacaataaa aagggatata cgtaatgtat tgtccttgag 600  
 agtgttggtg aacggaacat tctcaaaaca cccaatgga tacaagtcct tatcaactct 660  
 gcatagattg aatgcatatg accagagtgg aaggcttgtt gctaaacttg ttgctactga 720  
 tgatcttaca gtggaggatg aagaagatgg ccatcgatc ctcaactcac tcttcgagcg 780  
 tcttaatgaa ggacattcaa agccaattcg agcagctgaa actgcggtgg gagtcttatc 840  
 ccaatttggg caagagcacc gattatcacc agaagaggga gacaattaaa ctggtcacag 900

aagaacttta tcttttaagt aaaagaattg atgataacat attgttccac aaaacagtaa 960  
tagctaacag ctccataata gctgacatgg ttgtatcatt atcattatta gaaacattgt 1020  
atgaaatgaa ggatgtggtt gaagtgtaca gcaggcagtg cttgtgaatt taaaataaaa 1080  
atcctcttgt tactact 1097

<210> 27

<211> 770

<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 27

Met Thr Leu Ala Lys Ile Glu Leu Leu Lys Gln Leu Leu Arg Asp Asn  
1 5 10 15

Glu Ala Lys Thr Val Leu Lys Gln Thr Thr Val Asp Gln Tyr Asn Ile  
20 25 30

Ile Arg Lys Phe Asn Thr Ser Arg Ile Glu Lys Asn Pro Ser Leu Arg  
35 40 45

Met Lys Trp Ala Met Cys Ser Asn Phe Pro Leu Ala Leu Thr Lys Gly  
50 55 60

Asp Met Ala Asn Arg Ile Pro Leu Glu Tyr Lys Gly Ile Gln Leu Lys  
65 70 75 80

Thr Asn Ala Glu Asp Ile Gly Thr Lys Gly Gln Met Cys Ser Ile Ala  
85 90 95

Ala Val Thr Trp Trp Asn Thr Tyr Gly Pro Ile Gly Asp Thr Glu Gly  
100 105 110

Phe Glu Lys Val Tyr Glu Ser Phe Phe Leu Arg Lys Met Arg Leu Asp  
115 120 125

Asn Ala Thr Trp Gly Arg Ile Thr Phe Gly Pro Val Glu Arg Val Arg  
130 135 140

Lys Arg Val Leu Leu Asn Pro Leu Thr Lys Glu Met Pro Pro Asp Glu  
145 150 155 160

Ala Ser Asn Val Ile Met Glu Ile Leu Phe Pro Lys Glu Ala Gly Ile  
165 170 175

Pro Arg Glu Ser Thr Trp Ile His Arg Glu Leu Ile Lys Glu Lys Arg  
180 185 190

Glu Lys Leu Lys Gly Thr Met Ile Thr Pro Ile Val Leu Ala Tyr Met

195	200	205
Leu Glu Arg Glu Leu Val Ala Arg Arg Arg Phe Leu Pro Val Ala Gly		
210	215	220
Ala Thr Ser Ala Glu Phe Ile Glu Met Leu His Cys Leu Gln Gly Glu		
225	230	235 240
Asn Trp Arg Gln Ile Tyr His Pro Gly Gly Asn Lys Leu Thr Glu Ser		
	245	250 255
Arg Ser Gln Ser Met Ile Val Ala Cys Arg Lys Ile Ile Arg Arg Ser		
	260	265 270
Ile Val Ala Ser Asn Pro Leu Glu Leu Ala Val Glu Ile Ala Asn Lys		
	275	280 285
Thr Val Ile Asp Thr Glu Pro Leu Lys Ser Cys Leu Thr Ala Ile Asp		
	290	295 300
Gly Gly Asp Val Ala Cys Asp Ile Ile Arg Ala Ala Leu Gly Leu Lys		
305	310	315 320
Ile Arg Gln Arg Gln Arg Phe Gly Arg Leu Glu Leu Lys Arg Ile Ser		
	325	330 335
Gly Arg Gly Phe Lys Asn Asp Glu Glu Ile Leu Ile Gly Asn Gly Thr		
	340	345 350
Ile Gln Lys Ile Gly Ile Trp Asp Gly Glu Glu Glu Phe His Val Arg		
	355	360 365
Cys Gly Glu Cys Arg Gly Ile Leu Lys Lys Ser Lys Met Arg Met Glu		
	370	375 380
Lys Leu Leu Ile Asn Ser Ala Lys Lys Glu Asp Met Lys Asp Leu Ile		
385	390	395 400
Ile Leu Cys Met Val Phe Ser Gln Asp Thr Arg Met Phe Gln Gly Val		
	405	410 415
Arg Gly Glu Ile Asn Phe Leu Asn Arg Ala Gly Gln Leu Leu Ser Pro		
	420	425 430
Met Tyr Gln Leu Gln Arg Tyr Phe Leu Asn Arg Ser Asn Asp Leu Phe		
	435	440 445
Asp Gln Trp Gly Tyr Glu Glu Ser Pro Lys Ala Ser Glu Leu His Gly		

450                      455                      460  
 Ile Asn Glu Leu Met Asn Ala Ser Asp Tyr Thr Leu Lys Gly Val Val  
 465                      470                      475                      480  
 Val Thr Lys Asn Val Ile Asp Asp Phe Ser Ser Thr Glu Thr Glu Lys  
                     485                      490                      495  
 Val Ser Ile Thr Lys Asn Leu Ser Leu Ile Lys Arg Thr Gly Glu Val  
                     500                      505                      510  
 Ile Met Gly Ala Asn Asp Val Ser Glu Leu Glu Ser Gln Ala Gln Leu  
                     515                      520                      525  
 Met Ile Thr Tyr Asp Thr Pro Lys Met Trp Glu Met Gly Thr Thr Lys  
                     530                      535                      540  
 Glu Leu Val Gln Asn Thr Tyr Gln Trp Val Leu Lys Asn Leu Val Thr  
 545                      550                      555                      560  
 Leu Lys Ala Gln Phe Leu Leu Gly Lys Glu Asp Met Phe Gln Trp Asp  
                     565                      570                      575  
 Ala Phe Glu Ala Phe Glu Ser Ile Ile Pro Gln Lys Met Ala Gly Gln  
                     580                      585                      590  
 Tyr Ser Gly Phe Ala Arg Ala Val Leu Lys Gln Met Arg Asp Gln Glu  
                     595                      600                      605  
 Val Met Lys Thr Asp Gln Phe Ile Lys Leu Leu Pro Phe Cys Phe Ser  
                     610                      615                      620  
 Pro Pro Lys Leu Arg Ser Asn Gly Glu Pro Tyr Gln Phe Leu Arg Leu  
 625                      630                      635                      640  
 Val Leu Lys Gly Gly Gly Glu Asn Phe Ile Glu Val Arg Lys Gly Ser  
                     645                      650                      655  
 Pro Leu Phe Ser Tyr Asn Pro Gln Thr Glu Val Leu Thr Ile Cys Gly  
                     660                      665                      670  
 Arg Met Met Ser Leu Lys Gly Lys Ile Glu Asp Glu Glu Arg Asn Arg  
                     675                      680                      685  
 Ser Met Gly Asn Ala Val Leu Ala Gly Phe Leu Val Ser Gly Lys Tyr  
                     690                      695                      700  
 Asp Pro Asp Leu Gly Asp Phe Lys Thr Ile Glu Glu Leu Glu Lys Leu



705                      710                      715                      720  
 Lys Pro Gly Glu Lys Ala Asn Ile Leu Leu Tyr Gln Gly Lys Pro Val  
                          725                      730                      735  
 Lys Val Val Lys Arg Lys Arg Tyr Ser Ala Leu Ser Asn Asp Ile Ser  
                          740                      745                      750  
 Gln Gly Ile Lys Arg Gln Arg Met Thr Val Glu Ser Met Gly Trp Ala  
                          755                      760                      765  
 Leu Ser  
                          770

&lt;210&gt; 28

&lt;211&gt; 752

&lt;212&gt; PRT

&lt;213&gt; Influenza B/Vienna/1/99/ca

&lt;400&gt; 28

Met Asn Ile Asn Pro Tyr Phe Leu Phe Ile Asp Val Pro Ile Gln Ala  
   1                      5                      10                      15  
 Ala Ile Ser Thr Thr Phe Pro Tyr Thr Gly Val Pro Pro Tyr Ser His  
                          20                      25                      30  
 Gly Thr Gly Thr Gly His Thr Ile Asp Thr Val Ile Arg Thr His Glu  
                          35                      40                      45  
 Tyr Ser Asn Lys Gly Lys Gln Tyr Val Ser Asp Ile Thr Gly Cys Thr  
                          50                      55                      60  
 Met Val Asp Pro Thr Asn Gly Pro Leu Pro Glu Asp Asn Glu Pro Ser  
   65                      70                      75                      80  
 Ala Tyr Ala Gln Leu Asp Cys Val Leu Glu Ala Leu Asp Arg Met Asp  
                          85                      90                      95  
 Glu Glu His Pro Gly Leu Phe Gln Ala Ala Ser Gln Asn Ala Met Glu  
                          100                      105                      110  
 Ala Leu Met Val Thr Thr Val Asp Lys Leu Thr Gln Gly Arg Gln Thr  
                          115                      120                      125  
 Phe Asp Trp Thr Val Cys Arg Asn Gln Pro Ala Ala Thr Ala Leu Asn  
   130                      135                      140

Thr Thr Ile Thr Ser Phe Arg Leu Asn Asp Leu Asn Gly Ala Asp Lys  
 145 150 155 160  
 Gly Gly Leu Val Pro Phe Cys Gln Asp Ile Ile Asp Ser Leu Asp Lys  
 165 170 175  
 Pro Glu Met Thr Phe Phe Ser Val Lys Asn Ile Lys Lys Lys Phe Pro  
 180 185 190  
 Ala Lys Asn Arg Lys Gly Phe Leu Ile Lys Arg Ile Pro Met Lys Val  
 195 200 205  
 Lys Asp Arg Ile Ser Arg Val Glu Tyr Ile Lys Arg Ala Leu Ser Leu  
 210 215 220  
 Asn Thr Met Thr Lys Asp Ala Glu Arg Gly Lys Leu Lys Arg Arg Ala  
 225 230 235 240  
 Ile Ala Thr Ala Gly Ile Gln Ile Arg Gly Phe Val Leu Val Val Glu  
 245 250 255  
 Asn Leu Ala Lys Asn Ile Cys Glu Asn Leu Glu Gln Ser Gly Leu Pro  
 260 265 270  
 Val Gly Gly Asn Glu Lys Lys Ala Lys Leu Ser Asn Ala Val Ala Lys  
 275 280 285  
 Met Leu Ser Asn Cys Pro Pro Gly Gly Ile Ser Met Thr Val Thr Gly  
 290 295 300  
 Asp Asn Thr Lys Trp Asn Glu Cys Leu Asn Pro Arg Val Phe Leu Ala  
 305 310 315 320  
 Met Thr Glu Arg Ile Thr Arg Asp Ser Pro Ile Trp Phe Arg Asp Phe  
 325 330 335  
 Cys Ser Ile Ala Pro Val Leu Phe Ser Asn Lys Ile Ala Arg Leu Gly  
 340 345 350  
 Lys Gly Phe Met Ile Thr Ser Lys Thr Lys Arg Leu Lys Ala Gln Ile  
 355 360 365  
 Pro Cys Pro Asp Leu Phe Ser Ile Pro Leu Glu Arg Tyr Asn Glu Glu  
 370 375 380  
 Thr Arg Ala Lys Leu Lys Lys Leu Lys Pro Phe Phe Asn Glu Glu Gly  
 385 390 395 400

Thr Ala Ser Leu Ser Pro Gly Met Met Met Gly Met Phe Asn Met Leu  
 405 410 415  
 Ser Thr Val Leu Gly Val Ala Ala Leu Gly Ile Lys Asn Ile Gly Asn  
 420 425 430  
 Lys Glu Tyr Leu Trp Asp Gly Leu Gln Ser Ser Asp Asp Phe Ala Leu  
 435 440 445  
 Phe Val Asn Ala Lys Asp Glu Glu Thr Cys Met Glu Gly Ile Asn Asp  
 450 455 460  
 Phe Tyr Arg Thr Cys Lys Leu Leu Gly Ile Asn Met Ser Lys Lys Lys  
 465 470 475 480  
 Ser Tyr Cys Asn Glu Thr Gly Met Phe Glu Phe Thr Ser Met Phe Tyr  
 485 490 495  
 Arg Asp Gly Phe Val Ser Asn Phe Ala Met Glu Ile Pro Ser Phe Gly  
 500 505 510  
 Val Ala Gly Val Asn Glu Ser Ala Asp Met Ala Ile Gly Met Thr Ile  
 515 520 525  
 Ile Lys Asn Asn Met Ile Asn Asn Gly Met Gly Pro Ala Thr Ala Gln  
 530 535 540  
 Thr Ala Ile Gln Leu Phe Ile Ala Asp Tyr Arg Tyr Thr Tyr Lys Cys  
 545 550 555 560  
 His Arg Gly Asp Ser Lys Val Glu Gly Lys Arg Met Lys Ile Ile Lys  
 565 570 575  
 Glu Leu Trp Glu Asn Thr Lys Gly Arg Asp Gly Leu Leu Val Ala Asp  
 580 585 590  
 Gly Gly Pro Asn Ile Tyr Asn Leu Arg Asn Leu His Ile Pro Glu Ile  
 595 600 605  
 Val Leu Lys Tyr Asn Leu Met Asp Pro Glu Tyr Lys Gly Arg Leu Leu  
 610 615 620  
 His Pro Gln Asn Pro Phe Val Gly His Leu Ser Ile Glu Gly Ile Lys  
 625 630 635 640  
 Glu Ala Asp Ile Thr Pro Ala His Gly Pro Val Lys Lys Met Asp Tyr  
 645 650 655

Asp Ala Val Ser Gly Thr His Ser Trp Arg Thr Lys Arg Asn Arg Ser  
                   660                                665                                670  
  
 Ile Leu Asn Thr Asp Gln Arg Asn Met Ile Leu Glu Glu Gln Cys Tyr  
                   675                                680                                685  
  
 Ala Lys Cys Cys Asn Leu Phe Glu Ala Cys Phe Asn Ser Ala Ser Tyr  
                   690                                695                                700  
  
 Arg Lys Pro Val Gly Gln His Ser Met Leu Glu Ala Met Ala His Arg  
                   705                                710                                715                                720  
  
 Leu Arg Met Asp Ala Arg Leu Asp Tyr Glu Ser Gly Arg Met Ser Lys  
                                 725                                730                                735  
  
 Asp Asp Phe Glu Lys Ala Met Ala His Leu Gly Glu Ile Gly Tyr Thr  
                   740                                745                                750

&lt;210&gt; 29

&lt;211&gt; 726

&lt;212&gt; PRT

&lt;213&gt; Influenza B/Vienna/1/99/ca

&lt;400&gt; 29

Met Asp Thr Phe Ile Thr Arg Asn Phe Gln Thr Thr Ile Ile Gln Lys  
       1                                5                                10                                15  
  
 Ala Lys Asn Thr Met Ala Glu Phe Ser Glu Asp Pro Glu Leu Gln Pro  
                   20                                25                                30  
  
 Ala Met Leu Phe Asn Ile Cys Val His Leu Glu Val Cys Tyr Val Ile  
                   35                                40                                45  
  
 Ser Asp Met Asn Phe Leu Asp Glu Glu Gly Lys Ala Tyr Thr Ala Leu  
                   50                                55                                60  
  
 Glu Gly Gln Gly Lys Glu Gln Asn Leu Arg Pro Gln Tyr Glu Val Ile  
       65                                70                                75                                80  
  
 Glu Gly Met Pro Arg Thr Ile Ala Trp Met Val Gln Arg Ser Leu Ala  
                   85                                90                                95  
  
 Gln Glu His Gly Ile Glu Thr Pro Lys Tyr Leu Ala Asp Leu Phe Asp  
                   100                                105                                110

Tyr Lys Thr Lys Arg Phe Ile Glu Val Gly Ile Thr Lys Gly Leu Ala  
 115 120 125  
 Asp Asp Tyr Phe Trp Lys Lys Lys Glu Lys Leu Gly Asn Ser Met Glu  
 130 135 140  
 Leu Met Ile Phe Ser Tyr Asn Gln Asp Tyr Ser Leu Ser Asn Glu Ser  
 145 150 155 160  
 Ser Leu Asp Glu Glu Gly Lys Gly Arg Val Leu Ser Arg Leu Thr Glu  
 165 170 175  
 Leu Gln Ala Glu Leu Ser Leu Lys Asn Leu Trp Gln Val Leu Ile Gly  
 180 185 190  
 Glu Glu Asp Val Glu Lys Gly Ile Asp Phe Lys Leu Gly Gln Thr Ile  
 195 200 205  
 Ser Arg Leu Arg Asp Ile Ser Val Pro Ala Gly Phe Ser Asn Phe Glu  
 210 215 220  
 Gly Met Arg Ser Tyr Ile Asp Asn Ile Asp Pro Lys Gly Ala Ile Glu  
 225 230 235 240  
 Arg Asn Leu Ala Arg Met Ser Pro Leu Val Ser Val Thr Pro Lys Lys  
 245 250 255  
 Leu Lys Trp Glu Asp Leu Arg Pro Ile Gly Pro His Ile Tyr Asn His  
 260 265 270  
 Glu Leu Pro Glu Val Pro Tyr Asn Ala Phe Leu Leu Met Ser Asp Glu  
 275 280 285  
 Leu Gly Leu Ala Asn Met Thr Glu Gly Lys Ser Lys Lys Pro Lys Thr  
 290 295 300  
 Leu Ala Lys Glu Cys Leu Glu Lys Tyr Ser Thr Leu Arg Asp Gln Thr  
 305 310 315 320  
 Asp Pro Ile Leu Ile Met Lys Ser Glu Lys Ala Asn Glu Asn Phe Leu  
 325 330 335  
 Trp Lys Leu Trp Arg Asp Cys Val Asn Thr Ile Ser Asn Glu Glu Met  
 340 345 350  
 Ser Asn Glu Leu Gln Lys Thr Asn Tyr Ala Lys Trp Ala Thr Gly Asp  
 355 360 365

Gly Leu Thr Tyr Gln Lys Ile Met Lys Glu Val Ala Ile Asp Asp Glu  
 370 375 380  
 Thr Met Cys Gln Glu Glu Pro Lys Ile Pro Asn Lys Cys Arg Val Ala  
 385 390 395 400  
 Ala Trp Val Gln Thr Glu Met Asn Leu Leu Ser Thr Leu Thr Ser Lys  
 405 410 415  
 Lys Ala Leu Asp Leu Pro Glu Ile Gly Pro Asp Val Ala Pro Val Glu  
 420 425 430  
 His Val Gly Ser Glu Arg Arg Lys Tyr Phe Val Asn Glu Ile Asn Tyr  
 435 440 445  
 Cys Lys Ala Ser Thr Val Met Met Lys Tyr Val Leu Phe His Thr Ser  
 450 455 460  
 Leu Leu Asn Glu Ser Asn Ala Ser Met Gly Lys Tyr Lys Val Ile Pro  
 465 470 475 480  
 Ile Thr Asn Arg Val Val Asn Glu Lys Gly Glu Ser Phe Asp Met Leu  
 485 490 495  
 Tyr Gly Leu Ala Val Lys Gly Gln Ser His Leu Arg Gly Asp Thr Asp  
 500 505 510  
 Val Val Thr Val Val Thr Phe Glu Phe Ser Ser Thr Asp Pro Arg Val  
 515 520 525  
 Asp Ser Gly Lys Trp Pro Lys Tyr Thr Val Phe Arg Ile Gly Ser Leu  
 530 535 540  
 Phe Val Ser Gly Arg Glu Lys Ser Val Tyr Leu Tyr Cys Arg Val Asn  
 545 550 555 560  
 Gly Thr Asn Lys Ile Gln Met Lys Trp Gly Met Glu Ala Arg Arg Cys  
 565 570 575  
 Leu Leu Gln Ser Met Gln Gln Met Glu Ala Ile Val Glu Gln Glu Ser  
 580 585 590  
 Ser Ile Gln Gly Tyr Asp Met Thr Lys Ala Cys Phe Lys Gly Asp Arg  
 595 600 605  
 Val Asn Ser Pro Lys Thr Phe Ser Ile Gly Thr Gln Glu Gly Lys Leu  
 610 615 620

Val Lys Gly Ser Phe Gly Lys Ala Leu Arg Val Ile Phe Thr Lys Cys  
625 630 635 640

Leu Met His Tyr Val Phe Gly Asn Ala Gln Leu Glu Gly Phe Ser Ala  
645 650 655

Glu Ser Arg Arg Leu Leu Leu Leu Ile Gln Ala Leu Lys Asp Arg Lys  
660 665 670

Gly Pro Trp Val Phe Asp Leu Glu Gly Met Tyr Ser Gly Ile Glu Glu  
675 680 685

Cys Ile Ser Asn Asn Pro Trp Val Ile Gln Ser Ala Tyr Trp Phe Asn  
690 695 700

Glu Trp Leu Gly Phe Glu Lys Glu Gly Ser Lys Val Leu Glu Ser Val  
705 710 715 720

Asp Glu Ile Met Asp Glu  
725

<210> 30

<211> 584

<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 30

Met Lys Ala Ile Ile Val Leu Leu Met Val Val Thr Ser Asn Ala Asp  
1 5 10 15

Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val Lys  
20 25 30

Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Ala Ile Pro Leu Thr  
35 40 45

Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Lys Thr  
50 55 60

Arg Gly Lys Leu Cys Pro Thr Cys Leu Asn Cys Thr Asp Leu Asp Val  
65 70 75 80

Ala Leu Gly Arg Pro Met Cys Val Gly Ile Thr Pro Ser Ala Lys Ala  
85 90 95

Ser Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys Phe Pro Ile

100	105	110
Met His Asp Arg Thr Lys Ile Arg Gln Leu Pro Asn Leu Leu Arg Gly		
115	120	125
Tyr Glu Lys Ile Arg Leu Ser Thr Gln Asn Val Ile Asn Thr Glu Lys		
130	135	140
Ala Pro Gly Gly Pro Tyr Arg Leu Gly Thr Ser Gly Ser Cys Pro Asn		
145	150	155
Ala Thr Ser Lys Ser Gly Phe Phe Ala Thr Met Ala Trp Ala Val Pro		
165	170	175
Arg Asp Asn Asn Lys Thr Ala Thr Asn Pro Leu Thr Val Glu Val Pro		
180	185	190
His Ile Cys Thr Lys Glu Glu Asp Gln Ile Thr Val Trp Gly Phe His		
195	200	205
Ser Asp Asn Lys Thr Gln Met Lys Asn Leu Tyr Gly Asp Ser Asn Pro		
210	215	220
Gln Lys Phe Thr Ser Ser Ala Asn Gly Ile Thr Thr His Tyr Val Ser		
225	230	235
Gln Ile Gly Gly Phe Pro Asp Gln Thr Glu Asp Gly Gly Leu Pro Gln		
245	250	255
Ser Gly Arg Ile Val Val Asp Tyr Met Val Gln Lys Pro Gly Lys Thr		
260	265	270
Gly Thr Ile Val Tyr Gln Arg Gly Ile Leu Leu Pro Gln Lys Val Trp		
275	280	285
Cys Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser Leu Pro Leu Ile		
290	295	300
Gly Glu Ala Asp Cys Leu His Glu Lys Tyr Gly Gly Leu Asn Lys Ser		
305	310	315
Lys Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile Gly Asn Cys Pro		
325	330	335
Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg		
340	345	350
Pro Pro Ala Lys Leu Leu Lys Glu Arg Gly Phe Phe Gly Ala Ile Ala		



355                      360                      365  
 Gly Phe Leu Glu Gly Gly Trp Glu Gly Met Ile Ala Gly Trp His Gly  
 370                      375                      380  
 Tyr Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu Lys  
 385                      390                      395                      400  
 Ser Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser Leu  
 405                      410                      415  
 Ser Glu Leu Glu Val Asn Asn Leu Gln Arg Leu Ser Gly Ala Met Asp  
 420                      425                      430  
 Glu Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp Leu  
 435                      440                      445  
 Arg Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu Ser  
 450                      455                      460  
 Asn Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu Glu  
 465                      470                      475                      480  
 Arg Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Asp Ile Gly Asn  
 485                      490                      495  
 Gly Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp Arg  
 500                      505                      510  
 Ile Ala Ala Gly Thr Phe Asn Ala Glu Glu Phe Ser Leu Pro Thr Phe  
 515                      520                      525  
 Asp Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly Leu Asp  
 530                      535                      540  
 Asn His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser Leu Ala  
 545                      550                      555                      560  
 Val Thr Leu Met Ile Ala Ile Phe Ile Val Tyr Met Ile Ser Arg Asp  
 565                      570                      575  
 Asn Val Ser Cys Ser Ile Cys Leu  
 580

<210> 31  
 <211> 560  
 <212> PRT

&lt;213&gt; Influenza B/Vienna/1/99/ca

&lt;400&gt; 31

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Met Ser Asn Met Asp Ile Asp Gly Ile Asn Thr Gly Thr Ile Asp Lys
  1              5              10              15

Thr Pro Glu Glu Ile Thr Phe Gly Thr Ser Gly Thr Thr Arg Pro Ile
          20              25              30

Ile Arg Pro Ala Thr Leu Ala Pro Pro Ser Asn Lys Arg Thr Arg Asn
      35              40              45

Pro Ser Pro Glu Arg Ala Thr Thr Ser Ser Glu Ala Asp Val Gly Arg
      50              55              60

Lys Thr Gln Lys Lys Gln Thr Pro Thr Glu Ile Lys Lys Ser Val Tyr
      65              70              75              80

Asn Met Val Val Lys Leu Gly Glu Phe Tyr Asn Gln Met Met Val Lys
          85              90              95

Ala Gly Leu Asn Asp Asp Met Glu Arg Asn Leu Ile Gln Asn Ala His
          100              105              110

Ala Val Glu Arg Ile Leu Leu Ala Ala Thr Asp Asp Lys Lys Thr Glu
      115              120              125

Phe Gln Lys Lys Lys Asn Thr Arg Asp Val Lys Glu Gly Lys Glu Glu
      130              135              140

Ile Asp His Asn Lys Thr Gly Gly Thr Phe Tyr Lys Met Val Arg Asp
      145              150              155              160

Asp Lys Thr Ile Tyr Phe Ser Pro Ile Arg Ile Thr Phe Leu Lys Glu
          165              170              175

Glu Val Lys Thr Met Tyr Lys Thr Thr Met Gly Ser Asp Gly Phe Ser
          180              185              190

Gly Leu Asn His Ile Met Ile Gly His Ser Gln Met Asn Asp Val Cys
          195              200              205

Phe Gln Arg Ser Lys Ala Leu Lys Arg Val Gly Leu Asp Pro Ser Leu
      210              215              220

Ile Ser Thr Phe Ala Gly Ser Thr Ile Pro Arg Arg Ser Gly Ala Thr
      225              230              235              240

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Gly Val Ala Ile Lys Gly Gly Gly Thr Leu Val Ala Glu Ala Ile Arg  
 245 250 255  
 Phe Ile Gly Arg Ala Met Ala Asp Arg Gly Leu Leu Arg Asp Ile Lys  
 260 265 270  
 Ala Lys Thr Ala Tyr Glu Lys Ile Leu Leu Asn Leu Lys Asn Lys Cys  
 275 280 285  
 Ser Ala Pro Gln Gln Lys Ala Leu Val Asp Gln Val Ile Gly Ser Arg  
 290 295 300  
 Asn Pro Gly Ile Ala Asp Ile Glu Asp Leu Thr Leu Leu Ala Arg Ser  
 305 310 315 320  
 Met Val Val Val Arg Pro Ser Val Ala Ser Lys Val Val Leu Pro Ile  
 325 330 335  
 Ser Ile Tyr Ala Lys Ile Pro Gln Leu Gly Phe Asn Val Glu Glu Tyr  
 340 345 350  
 Ser Met Val Gly Tyr Glu Ala Met Ala Leu Tyr Asn Met Ala Thr Pro  
 355 360 365  
 Val Ser Ile Leu Arg Met Gly Asp Asp Ala Lys Asp Lys Ser Gln Leu  
 370 375 380  
 Phe Phe Met Ser Cys Phe Gly Ala Ala Tyr Glu Asp Leu Arg Val Leu  
 385 390 395 400  
 Ser Ala Leu Thr Gly Thr Glu Phe Lys Pro Arg Ser Ala Leu Lys Cys  
 405 410 415  
 Lys Gly Phe His Val Pro Ala Lys Glu Gln Val Glu Gly Met Gly Ala  
 420 425 430  
 Ala Leu Met Ser Ile Lys Leu Gln Phe Trp Ala Pro Met Thr Arg Ser  
 435 440 445  
 Gly Gly Asn Glu Val Gly Gly Asp Gly Gly Ser Gly Gln Ile Ser Cys  
 450 455 460  
 Ser Pro Val Phe Ala Val Glu Arg Pro Ile Ala Leu Ser Lys Gln Ala  
 465 470 475 480  
 Val Arg Arg Met Leu Ser Met Asn Ile Glu Gly Arg Asp Ala Asp Val  
 485 490 495

Lys Gly Asn Leu Leu Lys Met Met Asn Asp Ser Met Ala Lys Lys Thr  
 500 505 510

Ser Gly Asn Ala Phe Ile Gly Lys Lys Met Phe Gln Ile Ser Asp Lys  
 515 520 525

Asn Lys Thr Asn Pro Val Glu Ile Pro Ile Lys Gln Thr Ile Pro Asn  
 530 535 540

Phe Phe Phe Gly Arg Asp Thr Ala Glu Asp Tyr Asp Asp Leu Asp Tyr  
 545 550 555 560

<210> 32

<211> 100

<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 32

Met Asn Asn Ala Thr Phe Asn Tyr Thr Asn Val Asn Pro Ile Pro His  
 1 5 10 15

Ile Arg Gly Ser Val Ile Ile Thr Ile Cys Val Ser Phe Thr Val Ile  
 20 25 30

Leu Ile Ile Phe Gly Tyr Ile Ala Lys Ile Phe Thr Asn Arg Asn Asn  
 35 40 45

Cys Thr Asn Asn Ala Ile Gly Leu Cys Lys Arg Ile Lys Cys Ser Gly  
 50 55 60

Cys Glu Pro Phe Cys Asn Lys Arg Gly Asp Thr Ser Ser Pro Arg Thr  
 65 70 75 80

Gly Val Asp Ile Pro Ala Phe Ile Leu Pro Gly Leu Asn Leu Ser Glu  
 85 90 95

Ser Thr Pro Asn  
 100

<210> 33

<211> 466

<212> PRT

<213> Influenza B/Vienna/1/99/ca

&lt;400&gt; 33

Met Leu Pro Ser Thr Ile Gln Thr Leu Thr Leu Phe Leu Thr Ser Gly  
 1 5 10 15

Gly Val Leu Leu Ser Leu Tyr Val Ser Ala Ser Leu Ser Tyr Leu Leu  
 20 25 30

Tyr Ser Asp Ile Leu Leu Lys Phe Ser Pro Thr Glu Ile Thr Ala Pro  
 35 40 45

Thr Met Pro Leu Asp Cys Ala Asn Ala Ser Asn Val Gln Ala Val Asn  
 50 55 60

Arg Ser Ala Thr Lys Gly Val Thr Leu Leu Leu Pro Glu Pro Glu Trp  
 65 70 75 80

Thr Tyr Pro Arg Leu Ser Cys Pro Gly Ser Thr Phe Gln Lys Ala Leu  
 85 90 95

Leu Ile Ser Pro His Arg Phe Gly Glu Thr Lys Gly Asn Ser Ala Pro  
 100 105 110

Leu Ile Ile Arg Glu Pro Phe Ile Ala Cys Gly Pro Lys Glu Cys Lys  
 115 120 125

His Phe Ala Leu Thr His Tyr Ala Ala Gln Pro Gly Gly Tyr Tyr Asn  
 130 135 140

Gly Thr Arg Glu Asp Arg Asn Lys Leu Arg His Leu Ile Ser Val Lys  
 145 150 155 160

Leu Gly Lys Ile Pro Thr Val Glu Asn Ser Ile Phe His Met Ala Ala  
 165 170 175

Trp Ser Gly Ser Ala Cys His Asp Gly Lys Glu Trp Thr Tyr Ile Gly  
 180 185 190

Val Asp Gly Pro Asp Ser Asn Ala Leu Leu Lys Ile Lys Tyr Gly Glu  
 195 200 205

Ala Tyr Thr Asp Thr Tyr His Ser Tyr Ala Asn Asn Ile Leu Arg Thr  
 210 215 220

Gln Glu Ser Ala Cys Asn Cys Ile Gly Gly Asn Cys Tyr Leu Met Ile  
 225 230 235 240

Thr Asp Gly Ser Ala Ser Gly Ile Ser Glu Cys Arg Phe Leu Lys Ile

	245		250		255
Gln Glu Gly Arg Ile Ile Lys Glu Ile Phe Pro Thr Gly Arg Val Glu					
	260		265		270
His Thr Glu Glu Cys Thr Cys Gly Phe Ala Ser Asn Lys Thr Ile Glu					
	275		280		285
Cys Ala Cys Arg Asp Asn Ser Tyr Thr Ala Lys Arg Pro Phe Val Lys					
	290		295		300
Leu Asn Val Glu Thr Asp Thr Ala Glu Ile Arg Leu Met Cys Thr Glu					
305		310		315	320
Thr Tyr Leu Asp Thr Pro Arg Pro Asp Asp Gly Ser Ile Thr Gly Pro					
	325		330		335
Cys Glu Ser Asn Gly Asp Lys Gly Ser Gly Gly Ile Lys Gly Gly Phe					
	340		345		350
Val His Gln Arg Met Ala Ser Lys Thr Gly Arg Trp Tyr Ser Arg Thr					
	355		360		365
Met Ser Lys Thr Lys Arg Met Gly Met Gly Leu Tyr Val Lys Tyr Asp					
	370		375		380
Gly Asp Pro Trp Thr Asp Ser Asp Ala Leu Ala Leu Ser Gly Val Met					
385		390		395	400
Val Ser Met Glu Glu Pro Gly Trp Tyr Ser Phe Gly Phe Glu Ile Lys					
	405		410		415
Asp Lys Lys Cys Asp Val Pro Cys Ile Gly Ile Glu Met Val His Asp					
	420		425		430
Gly Gly Lys Glu Thr Trp His Ser Ala Ala Thr Ala Ile Tyr Cys Leu					
	435		440		445
Met Gly Ser Gly Gln Leu Leu Trp Asp Thr Val Thr Gly Val Asn Met					
450		455		460	
Ala Leu					
465					

<210> 34  
 <211> 248  
 <212> PRT

&lt;213&gt; Influenza B/Vienna/1/99/ca

&lt;400&gt; 34

Met Ser Leu Phe Gly Asp Thr Ile Ala Tyr Leu Leu Ser Leu Thr Glu  
 1 5 10 15

Asp Gly Glu Gly Lys Ala Glu Leu Ala Glu Lys Leu His Cys Trp Phe  
 20 25 30

Gly Gly Lys Glu Phe Asp Leu Asp Ser Ala Leu Glu Trp Ile Lys Asn  
 35 40 45

Lys Arg Cys Leu Thr Asp Ile Gln Lys Ala Leu Ile Gly Ala Ser Ile  
 50 55 60

Cys Phe Leu Lys Pro Lys Asp Gln Glu Arg Lys Arg Arg Phe Ile Thr  
 65 70 75 80

Glu Pro Leu Ser Gly Met Gly Thr Thr Ala Thr Lys Lys Lys Gly Leu  
 85 90 95

Ile Leu Ala Glu Arg Lys Met Arg Arg Cys Val Ser Phe His Glu Ala  
 100 105 110

Phe Glu Ile Ala Glu Gly His Glu Ser Ser Ala Leu Leu Tyr Cys Leu  
 115 120 125

Met Val Met Tyr Leu Asn Pro Gly Asn Tyr Ser Met Gln Val Lys Leu  
 130 135 140

Gly Thr Leu Cys Ala Leu Cys Glu Lys Gln Ala Ser His Ser His Arg  
 145 150 155 160

Ala His Ser Arg Ala Ala Arg Ser Ser Val Pro Gly Val Arg Arg Glu  
 165 170 175

Met Gln Met Val Ser Ala Met Asn Thr Ala Lys Thr Met Asn Gly Met  
 180 185 190

Gly Lys Gly Glu Asp Val Gln Lys Leu Ala Glu Glu Leu Gln Ser Asn  
 195 200 205

Ile Gly Val Leu Arg Ser Leu Gly Ala Ser Gln Lys Asn Gly Glu Gly  
 210 215 220

Ile Ala Lys Asp Val Met Glu Val Leu Lys Gln Ser Ser Met Gly Asn  
 225 230 235 240

Ser Ala Leu Val Lys Lys Tyr Leu  
245

<210> 35

<211> 109

<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 35

Met Leu Glu Pro Phe Gln Ile Leu Ser Ile Cys Ser Phe Ile Leu Ser  
1 5 10 15

Ala Leu His Phe Val Ala Trp Thr Ile Gly His Leu Asn Gln Ile Lys  
20 25 30

Arg Gly Val Asn Met Lys Ile Arg Ile Lys Ser Pro Asn Lys Glu Thr  
35 40 45

Ile Asn Arg Glu Val Ser Ile Leu Arg His Ser Tyr Gln Lys Glu Ile  
50 55 60

Gln Ala Lys Glu Thr Met Lys Glu Val Leu Ser Asp Asn Met Glu Val  
65 70 75 80

Leu Gly Asp His Ile Val Ile Glu Gly Leu Ser Ala Glu Glu Ile Ile  
85 90 95

Lys Met Gly Glu Thr Val Leu Glu Ile Glu Glu Leu His  
100 105

<210> 36

<211> 281

<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 36

Met Ala Asn Asn Ile Thr Thr Thr Gln Ile Glu Val Gly Pro Gly Ala  
1 5 10 15

Thr Asn Ala Thr Ile Asn Phe Glu Thr Gly Ile Leu Glu Cys Tyr Glu  
20 25 30

Arg Leu Ser Trp Gln Arg Ala Leu Asp Tyr Pro Gly Gln Asp Arg Leu  
35 40 45

Asn Arg Leu Lys Arg Lys Leu Glu Ser Arg Ile Lys Thr His Asn Lys



50				55				60							
Ser	Glu	Pro	Glu	Ser	Lys	Arg	Met	Ser	Leu	Glu	Glu	Arg	Lys	Ala	Ile
65					70					75					80
Gly	Val	Lys	Met	Met	Lys	Val	Leu	Leu	Phe	Met	Asn	Pro	Ser	Ala	Gly
				85					90					95	
Ile	Glu	Gly	Phe	Glu	Pro	Tyr	Tyr	Met	Lys	Ser	Ser	Ser	Asn	Ser	Asn
			100					105					110		
Cys	Pro	Lys	Tyr	Asn	Trp	Thr	Asp	Tyr	Pro	Ser	Thr	Pro	Gly	Arg	Cys
		115					120					125			
Leu	Asp	Asp	Ile	Glu	Glu	Glu	Pro	Glu	Asp	Val	Asp	Gly	Pro	Thr	Glu
	130					135					140				
Ile	Val	Leu	Arg	Asp	Met	Asn	Asn	Lys	Asp	Ala	Arg	Gln	Lys	Ile	Lys
145					150					155					160
Glu	Glu	Val	Asn	Thr	Gln	Lys	Glu	Gly	Lys	Phe	Arg	Leu	Thr	Ile	Lys
				165					170					175	
Arg	Asp	Ile	Arg	Asn	Val	Leu	Ser	Leu	Arg	Val	Leu	Val	Asn	Gly	Thr
			180					185					190		
Phe	Leu	Lys	His	Pro	Asn	Gly	Tyr	Lys	Ser	Leu	Ser	Thr	Leu	His	Arg
		195					200					205			
Leu	Asn	Ala	Tyr	Asp	Gln	Ser	Gly	Arg	Leu	Val	Ala	Lys	Leu	Val	Ala
	210					215					220				
Thr	Asp	Asp	Leu	Thr	Val	Glu	Asp	Glu	Glu	Asp	Gly	His	Arg	Ile	Leu
225					230					235					240
Asn	Ser	Leu	Phe	Glu	Arg	Leu	Asn	Glu	Gly	His	Ser	Lys	Pro	Ile	Arg
			245						250					255	
Ala	Ala	Glu	Thr	Ala	Val	Gly	Val	Leu	Ser	Gln	Phe	Gly	Gln	Glu	His
			260					265					270		
Arg	Leu	Ser	Pro	Glu	Glu	Gly	Asp	Asn							
		275					280								

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<210> 37
<211> 122
<212> PRT
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&lt;213&gt; Influenza B/Vienna/1/99/ca

&lt;400&gt; 37

Met Ala Asn Asn Ile Thr Thr Thr Gln Ile Glu Trp Arg Met Lys Lys  
 1 5 10 15

Met Ala Ile Gly Ser Ser Thr His Ser Ser Ser Val Leu Met Lys Asp  
 20 25 30

Ile Gln Ser Gln Phe Glu Gln Leu Lys Leu Arg Trp Glu Ser Tyr Pro  
 35 40 45

Asn Leu Val Lys Ser Thr Asp Tyr His Gln Lys Arg Glu Thr Ile Lys  
 50 55 60

Leu Val Thr Glu Glu Leu Tyr Leu Leu Ser Lys Arg Ile Asp Asp Asn  
 65 70 75 80

Ile Leu Phe His Lys Thr Val Ile Ala Asn Ser Ser Ile Ile Ala Asp  
 85 90 95

Met Val Val Ser Leu Ser Leu Leu Glu Thr Leu Tyr Glu Met Lys Asp  
 100 105 110

Val Val Glu Val Tyr Ser Arg Gln Cys Leu  
 115 120